EFFECTS OF ENERGY SOURCES ON MILK PRODUCTION AND REPRODUCTION OF DAIRY COWS

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DECLARATION

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ABSTRACT

Two main objectives were investigated in this study, conducted at the Elsenburg Research Farm of the Western Cape Department of Agriculture (WCDA). The first objective was to study the effect of nutritional treatments differing after calving in energy levels and sources on plasma metabolites and live weight (LW) changes, milk production and milk composition, ovarian follicular dynamics and oestrous activity, as well as fertility traits of dairy cows. The second objective was to investigate the effects of two late prepartum diets differing in non-fibre carbohydrates (NFC) and protein levels on milk production and milk composition, LW changes and fertility traits of dairy cows in the subsequent lactation. Three trials were conducted in this study. The first trial explored the effect of energy-dense ingredients like fat or starch on milk production and milk composition, plasma metabolites, LW change and fertility of lactating dairy cows. In this study, pregnant Holstein heifers and dry cows were similarly managed and fed prepartum. Following calving, they were allowed to graze *ad libitum* on kikuyu-ryegrass pasture, and received supplements that differed in terms of inclusion levels and types of concentrates. Holstein cows in the control group received 7 kg/day of a control concentrate supplement for both primiparous and multiparous groups, while treatments consisted of 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively. Supplements in treatments contained high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) levels. The control and HSLF concentrates were offered from calving to 154 days in milk (DIM). The HSLF supplement was a glucogenic concentrate containing maize as the energy source. The HSLF-LSHF supplements were combinations of a glucogenic concentrate offered for the first 60 DIM as per treatment HSLF and followed from 61 to 154 DIM by a lipogenic concentrate containing wheat bran and calcium (Ca)-salts of long-chain fatty acids as the energy sources. The postpartum diets were formulated to be isonitrogenous. The control diet contained low energy level (2.47 Mcal ME/kg DM) and provided 457, 104 and 40 g/kg of NDF, starch and fat on DM basis, respectively. The HSLF diet contained high energy level (2.71 Mcal ME/kg DM) and offered 341, 242 and 35 g/kg of NDF, starch and fat on DM basis, respectively. The HSLF treatment was formulated to increase plasma insulin and glucose levels in order to reduce the magnitude of the NEB and encourage the early return of oestrous cyclicity after calving. In the HSLF-LSHF combination treatment, the high starch-based diet initially aimed to achieve the same objective of the HSLF treatment during the first 60 DIM. The LSHF diet contained high energy level (2.70 Mcal ME/kg DM) and provided 388, 137 and 58 g/kg of NDF, starch and fat on DM basis, respectively. Secondly, the LSHF diet was fed from 61 DIM to decrease plasma insulin and

improve plasma cholesterol in order to increase the size of the ovulatory follicle as well as promote embryo development.

The second experiment trial was simultaneously conducted with the first trial using the same feeding treatments. In this trial, the same objectives of the diets were evaluated for ovarian follicular dynamics and oestrous activity of multiparous cows after being synchronized by means of the Ovsynch protocol at 80 ± 10 DIM without artificial insemination (AI). Results on plasma metabolites showed that postpartum levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) did not differ between nutritional treatments for multiparous cows, but levels increased ($P < 0.05$) for the control in comparison to HSLF and HSLF-LSHF treatments for primiparous animals. In addition, plasma urea levels were higher ($P < 0.05$) in both primiparous and multiparous animals receiving HSLF and HSLF-LSHF concentrates, compared to the control concentrate. The postpartum LW was the lowest ($P < 0.05$) and LW loss was greater ($P < 0.05$) in cows in the control in comparison to HSLF and HSLF-LSHF treatments in both primiparous and multiparous cows. Findings on productive responses showed that the milk yields and 4 % fat corrected milk (FCM) yields of both primiparous and multiparous cows in HSLF and HSLF-HFLS treatments were higher ($P < 0.05$), compared to cows in the control. In the primiparous group, the HSLF diet enhanced ($P < 0.05$) milk protein content whereas HSLF and HSLF-HFLS diets resulted in increased milk lactose content ($P < 0.05$). In multiparous group, HSLF treatment increased ($P < 0.05$) milk lactose content. Results of ovarian follicular dynamics and oestrous activity showed that dimensions of the ovaries and the preovulatory follicle as well as numbers of follicles within different classes in dairy cows were similar between nutritional treatments. However grazing cows receiving high (12.6 kg/day) levels of concentrates in the HSLF and HSLF-LSHF treatments recorded a higher number of total follicles ($P < 0.05$), compared to those on the low (7 kg/day) level of concentrate in the control group. Results of reproductive performance showed no nutritional effects in both primiparous and multiparous cows on the interval calving to first service, the percentage of cows serviced for the first time within the 80 DIM, pregnancy rate at first service, number of services per conception, interval days open and the pregnancy rate at 100 DIM. However, the conception rate at 150 DIM in both primiparous and multiparous groups improved ($P < 0.05$) in cows fed HSLF and HSLF-LSHF concentrates, in comparison to the control.

The third trial was conducted to evaluate the effect of two late prepartum nutritional treatments on subsequent LW changes, milk production and milk composition, and fertility traits of Holstein dairy cows. Pregnant heifers and dry cows received a similar level and type of prepartum concentrate

associated either unchopped oat hay *ad libitum* (control) or *ad libitum* intake of a partial total mixed ration (pTMR, treatment). The pTMR consisted of oat hay (48%), lucerne hay (43%) and soybean oil cake meal (9%). The prepartum treatment was designed to optimise rumen digestion and fermentation through improved prepartum intake of NFC and protein per kg on DM basis with an objective of reducing the extent of a periparturient negative energy balance (NEB) in comparison to the control. Following parturition, primiparous and multiparous cows received 7 kg/day of concentrate while grazing *ad libitum* on kikuyu-ryegrass pastures from calving to 120 DIM. Results showed that prepartum LW was similar for both primiparous and multiparous groups. In the primiparous group, the prepartum treatment significantly improved ($P < 0.05$) postpartum LW, with 20 kg more weight at LW nadir, compared to the control. However, no prepartum nutritional effects were detected on postpartum LW traits and LW loss for multiparous cows. Milk yields and milk composition were similar between the control and treatment for both primiparous and multiparous groups. Primiparous cows in the control group had a longer interval from calving to first service ($P < 0.05$), compared to those in treatment group. However, this improvement did not turn into increased conception rates of primiparous cows at 120 DIM. Similarly, prepartum diet effects in multiparous cows showed no postpartum differences in conception rates at 120 DIM.

It can concluded that there is definite merit in the use of energy levels and sources for improving production responses, plasma metabolite profiles and LW change, ovarian follicular dynamics and fertility of grazing dairy cows. These improvements are related to nutrient composition and levels of total energy intake, controlling the profile of nutrients absorbed in the gastrointestinal tract (GIT) and their hepatic partitioning to various body tissues for metabolic requirements of dairy cows. This modulation has probably influenced the hypothalamic-pituitary-ovarian-uterine axis through improved nutritional status and optimized the energy carry-over effect into fertility success, while sustaining milk production. However, feeding fibre-based diets differing in NFC and protein levels in the late prepartum period showed no benefit in either milk production or conception rates of dairy cows in the subsequent lactation. The lack of significant differences of prepartum dietary effects on milk production and conception success can be related to the prepartum feeding period and the relatively low number of animals per treatment used in the study. Further investigations should be undertaken using different inclusion and types of energy sources in prepartum and postpartum dairy cows on metabolic and hormonal responses that can improve restoration body reserves and benefit chronological sequences of the reproductive process such as postpartum health, uterine regression, resumption of oestrous cycles, conception, and embryo/pregnancy survival until the next calving.

OPSOMMING

Hierdie studie is uitgevoer op die Elsenburg Navorsingsplaas van die Wes-Kaapse Departement van Landbou (WKDL) en het twee hoofdoelwitte gehad. Die eerste doelwit was om die effek te bepaal van verskilllende voedingspeile, naamlik twee energiepeile en twee energiebronne, op spesifieke bloedplasma metaboliete, die verandering in liggaamsmassa, melkproduksie en -samestelling, ovarium en follikulêre aktiwiteite asook vrugbaarheidseienskappe van lakterende Holsteinkoeie wat die diete vanaf na kalwing ontvang het. Die tweede doelwit was om die effek te bepaal van twee diete, verskillend ten opsigte van nie-vesel koolhidraat- (NVK) en proteïenpeile, wat tydens die laat-dragtige periode aan melkkoeie gevoer is op die melkproduksie en -samestelling, verandering in liggaamsmassa en vrugbaarheidseienskappe van melkkoeie in die daaropvolgende laktasie. Vir die eerste doelwit is drie studies uitgevoer. In die eerste studie is die effek van energieryke grondstowwe soos vet of stysel op die melkproduksie en -samestelling, plasma metaboliete, liggaamsmassaverandering en die vrugbaarheid van lakterende melkkoeie bepaal. In hierdie studie is dragtige Holsteinverse en droë koeie voor kalwing op dieselfde manier bestuur en gevoer. Na kalwing het koeie *ad libitum* toegang gehad tot aangeplante kikoejoe-raaigrasweiding met kragvoeraanvullings wat verskil het ten opsigte van insluitingspeil en die tipe energiebron. Die kontrole-dieet het bestaan uit ‘n lae kragvoerpeil (7 kg/koei/dag) van ‘n standaard aanvulling terwyl behandelings bestaan het uit aanvullings wat (1) hoë stysel en lae vetpeile (HSLV) en (2) hoë stysel en lae vetpeile gevolg deur lae stysel en hoë vetpeile (HSLV-LSHV) bevat het. Dié aanvullings is teen hoër peile (11.6 en 12.4 kg/koei/dag vir eerste- en tweede-pluslaktasiekoeie, onerskeidelik) as die standaard kragvoer aanvulling voorsien. Die HSLV-aanvulling het bestaan uit ‘n konsentraat wat glukose bevat met mielies as energiebron en is vanaf kalwing tot 154 dae na kalf aan koeie voorsien. Die aanvullings wat in die behandeling HSLV-LSHV gebruik is, het bestaan uit kombinasies van ‘n glukose-bevattende energiebron vir die eerste 60 dae van die laktasieperiode, soortgelyk aan die behandeling HSLV, gevolg deur ‘n kragvoer (LSHV) wat vet bevat het as energiebron (koringsemels en kalsiumsoute met langkettingvetsure) wat vanaf 61 tot 154 dae na kalf aan koeie gevoer is.

Die tweede proef is gesamentlik met die eerste proef gedoen met dieselfde diete. Koeie wat op 80 ± 10 dae na kalf nog nie kunsmatig geïnsemineer was nie, is gesinchroniseer volgens die Ovsynch program en die follikulêre veranderinge en hitte-aktiwiteit van koeie is bepaal. Die energie-balansstudie het aangetoon dat geen verskille voorgekom het ten opsigte van die nie-esterbevattende vetsure (NEVS), plasma ureumpeile en liggaamsmassaveranderinge vir beide eerste- en

tweedeplus-laktasiekoeie nie. Die NEVS en β-hidroksiebutiraat (BHB) peile na-kalwing is ook nie beïnvloed vir die tweede-pluslaktasiekoeie nie terwyl die peile hoër ($P < 0.05$) was by eerstelaktasiekoeie wat die kontrole diet ontvang het in vergelyking met koeie wat die HSLV en HSLV-LSHV aanvullings ontvang het. Ureumpeile in bloedplasma in beide die eerste- en tweedeplus-laktasiekoeie is eweneens beïnvloed ($P < 0.05$) deur diëte met laer peile wat die kontrole dieet in vergelyking wat die HSLV en HSLV-LSHV aanvullings ontvang het. Aanvullend hierby het beide die eerste- en tweedeplus-laktasiekoeie wat die kontrole aanvulling ontvang het ‘n groter afname in liggaamsmassa ondervind in vergelyking met koeie wat die HSLV en HSLV-LSHV aanvullings ontvang het. Die produksiestudie het getoon dat die melkproduksie van koeie verhoog ($P < 0.05$) en dat sommige bestanddele in melk ook verander ($P < 0.05$) is deur die behandelings wat toegepas is. Die melkproduksie van beide eerstelaktasie- en ouer (tweede-pluslaktasie) koeie wat die HSLV en HSLV-LSHV behandelings ontvang het, was hoër as die melkproduksie van koeie wat die kontrole dieet ontvang het. Eerstelaktasiekoeie wat die HSLV aanvulling ontvang het, het melk met ‘n hoër ($P < 0.05$) proteienpersentasie geproduseer terwyl die melk van dié koeie wat die HSLV-LSHV aanvulling ontvang het, ‘n hoër ($P < 0.05$) laktosepersentasie bevat het. Netso het die melk van tweede-pluslaktasiekoeie wat die HSLV aanvulling ontvang het, hoër laktose peile bevat. Die follikulêre aktiwiteite in die ovaria en geslagsaktiwiteit is nie beïnvloed deur die vlak van energie-inname nie terwyl die totale aantal follikels wel beïnvloed ($P < 0.05$) is. Die reproduksieprestasie van beide eerste- en tweede-pluslaktasiekoeie vir die volgende reproduksie-eienskappe, naamlik periode vanaf kalf tot eerste inseminasie, die proporsie van koeie gedek voor 80 dae-in-melk, die persentasie besetting met eerste inseminasie, aantal dekkings per konsepsie, die aantal dae oop en die proporsie koeie wat beset was by 100 dae-in-melk, is nie beïnvloed deur kragvoeraanvullings nie. Die proporsie koeie wat beset was by 150 dae-in-melk is wel beïnvloed ($P < 0.05$) deur kragvoeraanvulling met meer eerstelaktasie- en tweedeplus-laktasie koeie beset wat die HSLV en HSLV-LSHV aanvullings ontvang het in vergelyking met die kontrole-aanvulling.

Die derde studie is uitgevoer om die invloed van die energiepeil in die dieet voor kalwing ten opsigte van liggaamsmassaverandering, melkproduksie en vrugbaarheidseienskappe gedurende die daaropvolgende laktasieperiode te bepaal. Dragtige verse en droë koeie is voor kalwing dieselfde tipe en hoeveelheid kragvoer voorsien tesame met (1) *ad libitum* gevoerde lang, ongekerfde hawerhooi en (2) ‘n *ad libitum* inname van ‘n gedeeltelike totaal gemengde rantsoen (pTMR) wat uit 48% hawerhooi, 43% lusernhooi en 9% sojaboon-oliekoekmeel bestaan het. Na kalwing is die eerstelaktasie- en tweedeplus-laktasiekoeie op aangeplante kikoejoe-raaigrasweiding aangehou en

het hulle dieselfde kragvoeraanvulling ontvang. Volgens die proefresultate het die voorkalwingsliggaamsmassa van beide eerstelaktasie- en tweedeplus-laktasiekoeie in die verskillende behandelingsgroepe nie verskil nie. Hierteenoor het die na-kalwingsliggaamsmassa van eerstelaktasiekoeie wat die kontrole-dieet (hawerhooi) en pTMR-voorkalwingsdieet ontvang het, verskil terwyl geen effek waargeneem is by die tweedeplus-laktasiekoeie nie. Die melkproduksie en –samestelling was dieselfde vir beide eerstelaktasie- en tweedeplus-laktasiekoeie wat die kontrole en behandelingsdiëte voorkalwing ontvang het. Eerstelaktasiekoeie wat net hawerhooi gedurende die voorkalwingsperiode ontvang het, het ‘n langer ($P < 0.05$) periode van kalf tot eerste inseminasie gehad in vergelyking met koeie wat die pTMR-dieet ontvang het. Geen verskille is gevind vir tweedeplus-laktasiekoeie nie. Net so is geen verskille tussen die kontrole en proefdiete gevind by beide eerstelaktasie- en tweedeplus-laktasiekoeie vir die persentasie koeie wat vir die eerste keer gedek is binne 80 dae na kalf, dragtigheid na eerste inseminasie, koeiliggaamsmassa met eerste dekking en besettingspersentasie teen 120 dae-in-melk.

Resultate toon dat daar beslis meriete bestaan om spesifieke energiebronne te gebruik om die volgende eienskappe te verbeter, naamlik melkproduksie, status van die enegiebalans en verandering in die liggaamsmassa van koeie asook follikulêre aktiwiteite, wys van hitte en die vrugbaarheid van koeie. Hierdie verbeteringe hou verband met voedingstofsamestelling en totale energie-innamevlakke, wat die profiel van voedingstowwe wat in die spysverteringskanaal (SVK) geabsorbeer word, sowel as die allokasie daarvan in die lewer na die onderskeie liggaamsweefsels vir die metaboliese behoeftes van melkkoeie, beheer. Die verandering in voedingstowwe het waarskynlik die hypothalamus-pituitêre-ovarium verbintenis beïnvloed tot ‘n beter energie balans met ‘n verdere oordragingseffek op die vrugbaarheid van koeie terwyl melkproduksiepeile gehandhaaf word. Tersefdertyd het ‘n verandering in die dieet wat dragtige verse en droëkoeie voorkalwing ontvang het geen voordele ingehou vir koeie in die daaropvolgende laktasieperiode ten opsigte van melkproduksie, -samestelling of besettingspersentasies nie. Dit kan waarskynlik toegeskryf word aan die beperkte aantal koeie wat in die proef in elke behandeling gebruik is. In hierdie studie het die behandelings nie-betekenisvolle produksie- en reproduksieresponse op alle gebiede getoon nie. Dit word aanbeveel dat die studie opgevolg behoort te word met groter getalle diere.

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DEDICATION

J'ai encore vu sous le soleil que la course n'est point aux agiles ni la guerre aux vaillants,
ni le pain aux sages, ni la richesse aux intelligents, ni la faveur aux savants;
car tout dépend pour eux du temps et des circonstances.
Ecclésiaste 9: 11

To my wife Rachel, my son Joshua and my daughter Amelia
To whom my success and progress are their main concerns,
To whom, by grace, makes the impossible possible,

I dedicate this work.

NOTES

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

CONFERENCE CONTRIBUTIONS

Oral presentations

Useni, B.A., Muller, C.J.C. & Cruywagen, C.W., 2014. Effect of energy sources on the milk production and reproduction of lactating Holstein cows. 6th All Africa Conference on Animal Agriculture, Nairobi, Kenya, 27-30 October 2014.

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Poster presentations

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Useni, B.A., Muller, C.J.C. & Cruywagen, C.W., 2015. Effect of different levels and types of energy sources on some fertility traits of Holstein cows. 48th SASAS Congress, 48th SASAS Congress, Empagneni, South Africa, 21-23 September, 2015.

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LIST OF ABBREVIATIONS

AI:	Artificial insemination
AOAC:	Association of Official Analytical Chemists
ATP:	Adenosine triphosphate
BCS:	Body condition score
BHB:	β-hydroxybutyrate
Ca:	Calcium
CIDR:	Controlled internal drug release
CP:	Crude protein
d:	Day
D:	Diameter
DIM:	Days in milk
DM:	Dry matter
DMI:	Dry matter intake
DP:	Dry period
EB:	Energy balance
EE:	Ether extract
FCM:	Fat corrected milk
GIT:	Gastro-intestinal tract
GH:	Growth hormone
GLM:	Generalised linear model
GnRH:	Gonadotropin releasing hormone
IGF-I:	Insulin-like growth factor-I
I-R:	Insulin receptor
HSLF:	High starch-low fat
LH:	Luteinizing hormone
LSHF:	Low starch-high fat
LW:	Live weight
ME:	Metabolizable energy
MPS:	Microbial protein synthesis
MUN:	Milk urea nitrogen
MY:	Milk yield
N:	Nitrogen

NFC: Non-fibre carbohydrate
NDF: Neutral detergent fibre
NEB: Negative energy balance
NEFA: Non-esterified fatty acids
NE_L: Net energy lactation
NFC: Non-fibre carbohydrate
NRC: National Research Council
NS: Not significant
P_4: Progesterone
P: Phosphor
P: Probability
PC: Pyruvate carboxylase
PEPCKm: Phosphoenolpyruvate carboxykinase-mitochondrial
RDP: Rumen degradable protein
RNA: Ribonucleic acid
$PGF_{2\alpha}$: Prostaglandin-$F_{2\alpha}$
pTMR: Partial total mixed ration
SAS: Statistical Analysis Systems
S: Season of calving
SD: Standard deviation
SEM: Standard errors of mean
ST: Interaction of season of calving and treatment
t: Time
T: Treatment
TAG: Tri-acyl glycerol
VFA: Volatile fatty acids
Y: Year
W: Week of observation
WCDA: Western Cape Department of Agriculture
WT: Interaction of week of observation and treatment

Chapter 1

General introduction

Over the past few decades, a significant increase in milk yield has been observed in dairy cows (Leroy *et al*., 2008a, b; Roche *et al*., 2011) because of an intense sire genetic selection, improved feeding of dairy cows while providing a better cow housing environment (Lucy, 2001; Thatcher *et al*, 2011). However, several studies have shown that these economically favourable improvements are associated with some negative physiological consequences, such as an increased incidence of metabolic (i.e. ketosis, fatty liver, lameness, etc.) and infectious diseases (i.e. mastitis, metritis), and a decline in fertility of dairy cows (Lucy, 2001, 2007; Butler, 2003; Walsh *et al*., 2011; Wathes, 2012). One of the major concerns of the physiological adaptation in mammals is that dry matter intake (DMI) does not meet the nutrient requirements during transition (e.g. at around calving), triggering a state of negative energy balance (NEB) (Butler, 2003; Ingvartsen, 2006; Roche *et al*., 2009). The state of NEB in dairy cows results in the mobilisation of body reserves (Rukkwamsuk *et al*., 1999) and the loss of LW (Jorristsma *et al*., 2003). In addition, it impairs the associated physiological and metabolic processes up to 10-12 weeks postpartum, and has been identified as an underlying factor of poor reproductive performance of dairy cows (Butler & Smith, 1989; Garnsworthy & Webb, 1999; Butler, 2003; Jorristsma *et al*., 2003). Failures in fertility have transpired to delayed resumption of ovarian activity (Staples *et al*., 1990), reduced oestrous expression (Lopez *et al*., 2004), diminished viability and quality of oocyte (Lucy *et al*., 1991), poor conception rates and more days open (Reist *et al*., 2003; Resken *et al*., 2002). Currently, failure of dairy cows to conceive is considered a key reason for culling, threatening the longevity of cows, as well as the sustainability of the dairy industry (De Vries, 2006).

During the postpartum period, different approaches to improve the reproductive performance in dairy cows have been put forward (Jorritsma *et al*. 2003; Lucy, 2007; Van Knegsel *et al*., 2007a, b; Garnsworthy *et al*., 2008a, b, c, d, 2009; Esposito *et al*., 2014). Most investigations found that improving the EB status during the transition period is of significance to ensure the subsequent well-being, milk production and fertility of dairy cows (Drackley *et al*., 2003). In this context, severe NEB during this periparturient period has been associated in the subsequent lactation to increased health problems (Duffield *et al*., 2009; Ospina *et al*., 2010a; McArt *et al*., 2012), decreased milk production and reduced reproductive success (Duffield *et al*., 2009; Ospina *et al*., 2010b, c). One suggested method, aiming for enhanced fertility while sustaining optimal milk yield, is to improve the energy density of the diet with non-fibre carbohydrates or fat (Staples *et al*., 1998;

Voigt *et al*., 2003; Gilmore *et al*., 2011). Dietary starch and/or fat increase the energy density of diets while reducing the severity of a NEB status (Van Knegsel *et al*., 2005). Dietary starch has been reported to improve insulin and glucose levels (Van Knegsel *et al*., 2007a, b; Garnsworthy *et al*., 2008b) and thus, have a positive influence on ovarian activity (Gong *et al*., 2002). However, a high level of dietary starch in the diet reduces the viability and quality of an oocyte and impairs the embryo developmental competence, thus leading to declining conception rates and a greater prevalence of embryo mortality (Armstrong *et al*., 2001; Leroy *et al*., 2008a, b). In contrast, dietary fat like calcium salts of palm fatty acids in the diet does not have inhibiting effects towards rumen microbial fermentation (Chalupa *et al*., 1986). Additionally, these fats were reported to enhance the growth and quality of follicles and also enhance progesterone (P_4) secretion, supporting an early embryo developmental competence (Gray *et al*., 2001; Wang *et al*., 2007).

Several studies have investigated the possible benefits of energy sources during the prepartum period on postpartum energy balance (EB), milk production and fertility of dairy cows. However, results are limited and in some instances conflicting, with some studies reporting a positive effect on EB (Grum *et al*., 1996; Janovick & Drackley, 2010; Janovick *et al*., 2011; Damgaard *et al*., 2013), milk yield (Ingvartsen & Andersen, 2000; Cavestany *et al*., 2009a), milk composition (Cavestany *et al*., 2009b; Grum *et al*., 1996; Damgaard *et al*., 2013), and fertility success (Frajblat & Butler, 2003; Cavestany *et al*., 2009b), while others studies showed no effects on these parameters (McNamara *et al*., 2003; Agenäs *et al*., 2003; Burke *et al*., 2010; Mann *et al*., 2015). Feeding energy as starch and/or fat *ad libitum* to dry cows enhanced DMI, allowing them to over consume energy relative to their demands, compared with those being fed a diet with a lower energy density (Janovick & Drackley, 2010). This overconsumption of energy was reported to be detrimental to the cow rumen health and the liver function (Beever, 2006), resulting in a greater decline in DMI (Minor *et al*., 1998; Olsson *et al*., 1998). Furthermore, dietary fat affected the EB status negatively, as evidenced by increased plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) levels and decreased plasma insulin levels during transition (Leroy *et al*., 2008b; Damgaard *et al*., 2013), which led to longer anoestrous periods (Giuliodori *et al*., 2011). In contrast, significant improvements of the cow health in early lactation have been reported in practice after feeding fibre-based diets containing > 400g/kg of NDF and low digestible energy levels on dry matter (DM) basis during the dry period (DP) (Drehmann, 2000; Beever, 2006; Jouany, 2006). Such diets were reported to optimise rumen microbial activity (Jouany, 2006), enhance metabolic status, reduce risks of ketosis and fatty liver syndrome (Janovick *et al*., 2011;

Vickers *et al*., 2013; Mann *et al*., 2015) and improve fertility outcomes in the subsequent lactation (Beever, 2006; Jouany, 2006).

Against this background, studies investigating the effect of energy sources and levels before and after calving to mid- or late lactation on reproductive performances of dairy cows are limited. Also, no studies have been conducted in South Africa to investigate the prepartum and/or postpartum effect of energy nutrients on milk responses and reproductive performances of dairy cows.

Appropriate nutrition programmes of dairy cows support not only the level of milk production but also ensure the rumen health, the restoration of body condition and energy stores between lactations, and the ability to calve at annually (Field & Taylor, 2012). The latter influences the culling rate, the size of the herd, the cow longevity and ultimately the financial return of lifetime milk production and the sustainability of dairy farming (De Vries, 2006; Leroy & De Kruif, 2006; Inchaisri *et al.* 2011). However, feed costs represent the largest single component (almost 70%) of the total input costs in dairy farming (Field & Taylor, 2012) and maintaining poor or non-fertile and/or lower milk producing cows in the dairy herd is obviously non-economical.

Trials were conducted at the Elsenburg Research Farm of the Western Cape Department of Agriculture (WCDA, Ethical clearance Project AP/BR/D/CM31). These trials evaluated the energy levels and sources in the diet of dairy cows before and/or after calving to improve fertility of Holstein cows, while sustaining milk production. The first trial investigated the effect of nutritional treatments differing after calving in energy levels (low *vs* high) and sources (starch *vs* fat) on plasma metabolites and LW changes, milk production and milk composition, and reproductive performances of Holstein cows until 154 days postpartum. The treatments were:

- Control: *ad libitum* pasture + control concentrate (7 kg/day for both primiparous and multiparous cows).
- Glucogenic Treatment: *ad libitum* pasture + high starch-low fat (HSLF) concentrate (11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively). The HSLF treatment was formulated to increase plasma insulin and glucose levels in order to reduce the extent of a NEB and improve an early resumption of oestrous activity and pregnancy rates in grazing cows.
- Glucogenic-lipogenic treatment: *ad libitum* pasture + high starch-low fat (HSLF) concentrates, followed from 61 days in milk (DIM) by low starch-high fat (LSHF) concentrate (11.6 and 12.6 kg/day of HSLF and LSHF concentrates for primiparous and multiparous cows, respectively). In addition to the HSLF effect during the first 60 DIM, the

LSHF concentrate was fed thereafter to decrease plasma insulin and increase plasma cholesterol in order to improve the ovulatory follicular size, the production of progesterone, and pregnancy rates in grazing cows.

The second trial was simultaneously conducted with the first trial and investigated the effect of the same nutritional treatments (i.e. control, HSLF and HSLF-LSHF supplements) on the ovarian follicular dynamics and oestrous activity of grazing multiparous cows. The third trial studied the 30 day effect of prepartum nutritional treatments differing in non-fibre carbohydrate (NFC) and protein levels on LW changes, milk production and milk composition, and fertility traits of Holstein cows until 120 days postpartum. The treatments were:

- Control: Prepartum: *ad libitum* unchopped oat hay + prepartum concentrate.
  Postpartum: *ad libitum* pasture + postpartum concentrate.

  The prepartum concentrate levels were fed from 30 to 14 days prepartum at 3 kg/day for both heifers and dry cows, and from 13 days prepartum to calving at 5 kg/day and 6 kg/day for heifers and dry cows, respectively. The postpartum concentrate was fed at 7 kg/day for both primiparous and multiparous cows.
- Treatment : Prepartum: *ad libitum* partial total mixed ration (pTMR, consisting of 48% oat hay + 43% lucerne hay and 9% soybean oil cake meal) + prepartum concentrate.
  Postpartum: *ad libitum* pasture + postpartum concentrate.

  The prepartum concentrate levels were fed from 30 to 14 days prepartum at 3 kg/day for both heifers and dry cows, and from 13 days prepartum to calving at 5 kg/day and 6 kg/day for heifers and dry cows, respectively. The postpartum concentrate was fed at 7 kg/day for both primiparous and multiparous cows. The prepartum treatment was formulated to optimise rumen digestion and fermentation, through improved prepartum intake of energy and protein per kg on DM basis, to reduce the extent of a periparturient NEB, compared to the control group.

The first hypothesis of this study was that manipulating the type and inclusion level of energy nutrients in the diet of dairy cows until 154 days postpartum can:

- influence the metabolite profiles and prevent a decrease in LW since the improved postpartum nutritional status [i.e. total nutrient intake and metabolizable energy (ME)] affects the extent of body fat mobilisation in the adipose tissues (Lafontan *et al.*, 2009), the nitrogen (N) metabolism (Moharrery, 2004.) as well as the restoration of LW (Bauman & Currie 1980);

- Improve milk yields and influence milk composition as made possible by an increase in the supply of milk precursors to the udder for milk synthesis (Van Knegsel *et al*., 2007a; Purushothaman *et al*., 2008; Hills *et al.*, 2015);
- Enhance ovarian follicular dynamics and oestrous activity in an *in vivo* systems as *in vitro* systems do not seem to provide the complete *in vivo* ovarian condition (Laskowski *et al*., 2016);
- Improve fertility traits in dairy cows, as the improved nutritional status and the carry-over effects of the positive EB affect positively the reproductive axis and improve fertility outcomes (Butler, 2003). Contrary to lipogenic conditions, high insulinogenic conditions from high starch-based diets were reported in *in vitro* systems to affect negatively the viability and quality of the oocyte and later of the embryo (Armstrong *et al*., 2001; Leroy *et al*., 2008a, b).

The second hypothesis of this study was that feeding fibre-based diets (> 400g/kg of NDF on DM basis) differing in NFC and protein levels during the DP can affect the energy and nutrient intake of periparturient dairy cows to subsequently minimise LW loss, influence milk production and milk composition, and improve the proportion of cows pregnant in the subsequent lactation as stated in some studies (Grum *et al*., 1996; Ingvartsen & Andersen, 2000; Cavestany *et al*., 2009a, b; Damgaard *et al*., 2013).

The first aim of this thesis was to investigate in a pasture-based system the effect of nutritional treatments differing after calving in energy levels (low *vs* high) and sources (starch *vs* fat) on:

1. Plasma metabolites and LW changes;
2. Milk production and milk composition;
3. Ovarian follicular dynamics and oestrous activity;
4. Fertility traits of Holstein cows.

The second aim of this thesis was to investigate the effect of two late prepartum fibre-based diets differing in levels (low *vs* high) of NFC and protein on:

5. Milk production and milk composition, LW changes and fertility traits of Holstein cows in the subsequent lactation in a pasture-based system.

With the prepartum and/or postpartum application of the energy levels and sources in the trials, it is expected that a nutritional contribution of this study can be made not only to enhance plasma

metabolite profiles and restore the postpartum LW of dairy cows but also to improve the milk responses, the resumption of the ovarian cyclicity, the oestrous behaviours during the breeding period, and the fertilisation success. These biological improvements increase the proportion of cows pregnant and the pregnancy survival until the next calving, while sustaining high milk production during the lactation period. Such enhancements to dairy farmers should ultimately result in the decrease in fertility related cullings and replacement of cows in the herd on one hand and the increase in herd size, cow longevity, lifetime milk production and financial return on the other hand.

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Chapter 2

Literature review

2.1. Introduction

Carbohydrates and fats are the major energy sources that are usually used in the diets of dairy cows (Schroeder *et al*., 2004; Carmo *et al*., 2015). Starch-based ingredients like cereals grains, non-fibre carbohydrates and propylene glycol provide the fermentable energy, and improve the protein/energy balance to potentially enhance the supply of rumen microbial protein synthesis (MPS) to the small intestine (Rearte & Pieroni, 2001; Bargo *et al*., 2003). Fat increases the energy density of the diet (Schroeder *et al*., 2004), and particularly enhances plasma cholesterol, the major precursor for luteal progesterone (P_4) synthesis in postpartum cows (Roche *et al*., 2011). Fat can be included in form of ruminally inert sources such as hydrogenated fish fat, high melting point fatty acids, and calcium (Ca)-salts of long-chain fatty acids or non-ruminally inert sources such as soybean oil and full fat rapeseed (Bargo *et al*., 2003). Manipulating the energy level and source in the diet of prepartum and/or postpartum cows showed significant improvements in terms of a decreased incidence of health problems, optimised rumen microbial activity, increased the amount of digested nutrients from the gastro-intestinal tract (GIT), decreased body condition loss, and improved fertility of dairy cows (Bauman & Currie 1980; Grum *et al*., 1996; Drehmann, 2000; Ingvartsen & Andersen, 2000; Butler *et al*., 2003; Beever, 2006; Jouany, 2006; Purushothaman *et al*., 2008; Lafontan *et al*., 2009; Cavestany *et al*., 2009a, b; Damgaard *et al*., 2013; Hills *et al*., 2015). Furthermore, modifying the inclusion level of either starch- or fat-based ingredients in postpartum diets has shown to improve milk yield in dairy cows (Van Knegsel *et al*., 2005; Reis *et al*., 2012; Roche *et al*., 2013). However, studies on the effects of either starch- or fat-based diets on dairy cow reproduction parameters are contradictory, with some studies reporting a negative or lack of an effect (Beam & Butler, 1997; Oldick *et al*., 1997; Garcia-Bojalil *et al*., 1998; Van Knegsel *et al*., 2007c; Dyck *et al*., 2011; Gilmore *et al*., 2011), compared to other studies reporting a positive effect (Gong *et al*., 2002; Garnsworthy *et al*., 2009; Burke *et al*., 2010; Reis *et al*., 2012). The objectives of this chapter are therefore to discuss the effects of energy-dense ingredients like non-fibre carbohydrates and/or fat during the dry period (DP) and postpartum period on the consequent metabolism, milk production and fertility of dairy cows.

2.2. Energy sources during the dry period

A lactation period of 305 days, followed by a DP of 56 to 60 days, has been considered as a strategic management system for most dairy farms since the 1950's (Bachman & Schairer, 2003). The DP is defined as a period of recovery following the lactating period of dairy cows (Beever, 2006). Dairy cows in the prepartum period require energy demands for body restoration and pregnancy maintenance (Jouany, 2006). While body growth is normal for primiparous cows during the DP, mature cows do not gain live weight (LW) to the same extent, except for the LW associated with the growth of the conceptus and foetal membranes (NRC, 2001). Despite an increased energy requirement, feed intake usually declines in the late DP (Grummer, 1995; Huzzey *et al*., 2007). The decrease in prepartum dry matter intake (DMI) can be attributed to hormonal, physiological and digestive factors related to this period, as well as by the rapid growth of the foetus which takes up abdominal space, thereby decreasing rumen volume (Jouany, 2006).

Several studies have investigated and/or reviewed the possible benefits of energy sources during the prepartum period (Grum *et al*., 1996; Drackley, 1999; Agenäs *et al*., 2003; McNamara *et al*., 2003; Dann *et al*., 2005; Janovick & Drackley, 2010; Janovick *et al*., 2011; Damgaard *et al*., 2013). However, results are limited and in some instances conflicting, with some studies showing a positive prepartum effect on energy balance (EB) status (Grum *et al*., 1996; Janovick & Drackley, 2010; Janovick *et al*., 2011; Damgaard *et al*., 2013), milk yield (Ingvartsen & Andersen, 2000; Cavestany *et al*., 2009a), milk composition (Cavestany *et al*., 2009b; Grum *et al*., 1996; Damgaard *et al*., 2013), reproduction performances (Cavestany *et al*., 2009b) and others reporting the lack of effect on these traits (McNamara *et al*., 2003; Agenäs *et al*., 2003; Burke *et al*., 2010; Mann *et al*., 2015). Unrestricted feeding of a DP diet with higher energy levels (starch or fat) to cows enhanced DMI, allowing cows to consume too much energy relative to their nutritional requirements, compared with those being fed a lower energy density diet (Janovick & Drackley, 2010). However, this overconsumption of energy was reported to be detrimental to the cow rumen health and liver function (Beever, 2006), resulting further in a greater decline in DMI during early lactation (Minor *et al*., 1998; Olsson *et al*., 1998). Feeding prepartum high starch diets increased the production of volatile fatty acids (VFA) and resulted in a decrease in pH and an accumulation of lactic acid. This decline in DMI and ruminal pH further exacerbates the EB deficit in cows (Jouany, 2006). Feeding high prepartum levels of dietary fat also negatively impacted the EB status, as evidenced by increased plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) levels and decreased insulin levels during the transition period (Leroy *et al*., 2008b; Damgaard *et al*., 2013),

which resulted in longer anoestrous periods (Giuliodori *et al*., 2011). In both energy feeding contexts, cows can possibly fail to adapt to the negative energy balance (NEB) stress when fed high prepartum levels of starch and/or fat due to the associated metabolic and rumen dysfunctions during the periparturient period (Jouany, 2006; Janovick *et al*., 2011; Mann *et al*., 2015). Furthermore, a severe NEB during the prepartum period has been associated in the subsequent lactation with increased health problems (Duffield *et al*., 2009; Ospina *et al*., 2010a; McArt *et al*., 2012), reduced reproductive success and decreased milk production (Duffield *et al*., 2009; Ospina *et al*., 2010b, c).

The optimal prepartum dietary management strategy with reference to control energy intake and/or DMI is still to be developed (Janovick & Drackley, 2010). Some studies have indicated that an overconsumption of energy prepartum is detrimental to postpartum cow health and liver function (Grum *et al*., 1996; Rukkwamsuk *et al*., 1999), whereas others demonstrated that supplementing extra energy during the DP is beneficial for transition success (Dann *et al*., 1999; Rabelo *et al*., 2005). Consequently, recent studies investigated the potential benefits of feeding fibre-based diets containing > 400g/kg of NDF and low digestible energy levels on DM basis during the DP (Janovick *et al*., 2011; Vickers *et al*., 2013; Mann *et al*., 2015). Such prepartum diets were reported to adjust the DMI, which optimised the rumen digestion and fermentation (Jouany, 2006) and decreased the mobilization of body reserves, as well as the deposition of lipid and tri-acyl glycerol (TAG) in the liver (Mann *et al*., 2015). Controlling the energy content in prepartum diets is usually achieved by adding bulky, lower quality forages such as chopped wheat straw or oat hay that increases fibre content and limits the voluntary DMI (NRC, 2001) thereby regulating the total nutrient consumption. This strategy of feeding fibre-based diets containing low digestible energy levels to dry cows has shown significant improvements in dairy herds in terms of fewer health problems, reduced body condition loss, and enhanced reproductive axis after parturition (Drehmann, 2000, Beever, 2006; Jouany, 2006). Thus, existing evidence revealed that feeding a prepartum forage-based diet containing low digestible energy level optimised rumen microbial activity (Jouany, 2006), improved metabolic status and reduced the risks of ketosis and fatty liver syndrome in periparturient dairy cows (Janovick *et al*., 2011; Vickers *et al*., 2013; Mann *et al*., 2015). However, feeding trials that investigated the prepartum effect of energy levels and sources on the postpartum milk responses and reproductive performances of dairy cows are limited, thus making it difficult to draw final conclusions.

2.3. Negative energy balance and postpartum related disorders in dairy cows

Over the past few decades, a significant increased milk yield has been observed in dairy cows (Leroy *et al*., 2008b, c; Roche *et al*., 2011) as a result of an intense genetic selection, improved dairy nutrition and better cow management (Lucy, 2001; Thatcher *et al*, 2011). However, several studies have shown that the improvement in milk yield is associated with some negative consequences, such as an increased occurrence of metabolic and infectious diseases and a decline in reproductive performance (Lucy, 2001, 2007; Butler, 2003; Walsh *et al*., 2011; Wathes, 2012). As indicators of reproduction management efficiency, both the calving interval and the number of artificial insemination (AI) per conception have increased substantially worldwide (Butler, 1998). In South African Holsteins for instance, the intercalving period increased from 386 days in 1986 to 412 days in 2004 (Makgahlela, 2008).

During the transition period from a pregnant, non-lactating state to a non-pregnant lactating state, dairy cows are confronted by numerous physiological challenges and stressors related to the parturition and the onset of lactation (Evans & Walsh, 2012; McArt *et al*., 2013; Esposito *et al*., 2014). One of the main challenges is a rapid rise in nutrient requirements (Ingvartsen, 2006), essentially doubling overnight once milk production begins (Drackley *et al*., 2005). In the week preceding calving, the cow's appetite decreased (Walsh *et al*., 2011) and the DMI has been reported to decline by approximately 30%, occurring in the 24 hours before calving (Huzzey *et al*., 2007). As a result, cows enter into a NEB status because of the increased energy demands from the onset of lactation until the beginning the breeding season (Butler, 2003; Roche *et al*., 2009). In response to the NEB, cows mobilize stored triglycerides from the adipose tissue in an attempt to meet the energy requirements for maintenance and milk production (Rukkwamsuk *et al*., 1999). The NEB impairs the general metabolic system in dairy cows and has been identified as an underlying causal factor of poor reproductive performance by a number of researchers (Butler & Smith, 1989; Garnsworthy & Webb, 1999; Butler, 2003; Jorritisma *et al*., 2003).

Several reviews have been published regarding the effect of the EB status in dairy cattle on reproductive efficiency (Beam & Butler, 1999; Butler, 2000, 2003; Jorritisma *et al*., 2003; Van Knegsel *et al*, 2005; Wathes *et al*., 2007; Santos *et al*., 2008; Roche *et al*., 2011; Evans & Walsh, 2012; Leroy *et al*., 2014). A status of NEB alters the insulin level and the insulin-like growth factor-I (IGF-I) - growth hormone (GH) axis to decrease the bioavailability of circulating IGF-I (Wathes *et al*., 2007). Furthermore, it decreases the luteinizing hormone (LH) pulse frequency, the diameter and growth rate of the dominant follicle, the activity of the corpus luteum, as well as the

perioestrous hormone levels like oestradiol and P_4 (Beam & Butler, 1997, 1999; Butler, 2000). The effects of these EB-induced alterations on fertility have resulted in increased number of days from calving to the resumption of oestrus and days open as well as to decreased conception rates following fertilisation and pregnancy rates thereafter (Giuliodori *et al*., 2011; Roche *et al*., 2011).

When dairy cows experience a NEB, their immune system is likely to be compromised (Mallard *et al*., 1998). The level of impairment and the degree of reclamation of the postpartum immune competence are strongly influenced by the extent and duration of the NEB around calving (Pyörälä, 2008; Wathes *et al*., 2009), making cows in a severe NEB more vulnerable to infections caused by pathogenic organisms (Goff, 2006; Wathes, 2012). Gröhn *et al*. (1995) studied the prevalence of postpartum diseases in multiparous cows in 25 Holstein herds in North America and found 7.4% incidence of retained placenta, 7.6% incidence of metritis and 4.9% incidence of ketosis, respectively. Jordan and Fourdraine (1993) surveyed 61 top milk producing herds in North America and reported 3.7% incidence of ketosis, 9.0% incidence of retained placenta and 12.8% incidence of metritis, respectively. Other reports found that the effects of metabolic biomarkers (i.e. high NEFA and BHB) due to poor adaptation of lactating cows to the energy stress were associated with the occurrence of abomasum displacement, clinical ketosis, lameness, mastitis, and endometritis, which all can contribute to an increased risk of culling of affected animals (Seifi *et al*., 2011; Walsh *et al*., 2011; Evans & Walsh, 2012; Esposito *et al*., 2014). Metabolic and infectious diseases can lead to lower milk yields (Rajala-Schultz *et al*., 1999a, b), lower conception rates (LeBlanc *et al*., 2002; Hansen *et al*., 2004; Bisinotto *et al*., 2012) and increased incidences of involuntary culling (Gröhn *et al*., 1998; Esposito *et al*., 2014).

2.4. Energy partitioning in dairy cows

Feed constituents such as dietary fibre, carbohydrates and protein provide substrates for rumen microbial fermentation that yields rumen volatile fatty acids (VFA), with the main ones being acetate, butyrate and propionate. Fat is hydrolysed into fatty acids and hydrogenated in the rumen. Ruminal bypass nutrients and microbial matter can be digested and absorbed in the small intestine, providing additional glucogenic and/or lipogenic compounds for the animal (McDonald *et al*., 2002). These absorbed nutrients proceed through a succession of pathway reactions of the Krebs cycle involving oxygen (respiratory chain reactions) to produce the body energy fuel as adenosine triphosphate (ATP) (Van Knegsel *et al*., 2005).

As parturition occurs and dairy cows shift into producing milk, the requirement of nutrients increases because of the onset of milk production and also of the initial depression of DMI (Walsh

et al., 2011; Evans & Walsh, 2012). Requirements for glucose and metabolizable energy (ME) increase by two- to three-fold after the onset of the lactation (Drackley *et al*., 2001). Also, an increase in postpartum plasma GH levels occurs, thus prioritizing high milk synthesis in the mammary gland (Chagas *et al*., 2007). In the liver, the improvement in plasma GH levels directly stimulates gluconeogenesis and indirectly antagonises the production of insulin, necessary for meeting glucose demands for milk production (Lucy, 2004). As a result of low plasma glucose and insulin levels, body fat and to a lesser degree body protein stored as body reserves, are mobilised (Van Knegsel *et al*., 2005), usually through homeostatic regulation (Roche *et al*., 2009; Thatcher *et al*., 2011). This mobilisation results in a loss of body condition score (BCS) and LW (Jorritsma *et al*., 2003; Van Straten *et al*., 2008) as a physiological mechanism to overcome the energy deficit. Non-esterified fatty acids are consequently released from body fat reserves, with increasing NEFA levels in the bloodstream suggesting an EB shortfall (Duffield, 2000, Wathes *et al*., 2007). The NEFA metabolites are either directed into the mammary gland to supply milk triglycerides (Drackley, 2000) or utilized in the liver (Drackley *et al*., 2001; Vernon, 2002; Schulz *et al*., 2014). Following their uptake by the liver, NEFA can be utilised in three pathways. Firstly, NEFA can be oxidized to carbon dioxide to supply energy as alternative energy fuel for other tissues while most of the glucose is diverted for lactose synthesis in the mammary gland (Vernon, 2002). Secondly, it can be partially oxidized to produce ketone bodies, acetone, aceto-acetate and BHB, which may result in ketosis (Schulz *et al*., 2014; Esposito *et al*., 2014). Thirdly, it may be esterified to triglycerides or phospholipids and stored in the liver as TAG, with the possibility of causing fatty liver syndrome (Drackley *et al*., 2001). This mobilisation highlights that the metabolic effects of a NEB status in early lactation induce an imbalance in the ratio of plasma glucogenic and lipogenic compounds derived from feed nutrients and body reserves (Schulz *et al*., 2014). Hence, the physiological consequences of postpartum EB deficit caused low plasma glucose and insulin levels associated with high levels of plasma NEFA, BHB, acetone, acetoacetate and liver TAG (Van Knegsel *et al*., 2005; Evans & Walsh, 2012). As lactating cows enter a state of NEB, they physiologically direct the limited available nutrients in their system to milk synthesis for the survival of living offspring. This prioritisation occurs at the expense of the reproductive axis, thus limiting the dominant follicle to ovulate, be fertilized and cared for during an entire gestation (Leroy *et al*., 2008a). From this brief review, it appears that the brain, the GIT, the body reverses, the foetus (before calving), and the udder as well as the reproductive organ (after calving) are all components of the adaptation to EB status in dairy cows. In addition, the liver obviously plays a key role in coordinating metabolic responses in dairy cows in order to adapt and recover from NEB.

Several studies have indicated that dietary energy sources can be manipulated through inclusion of feedstuffs in the diet to prevent and/or treat the NEB related disorders (Staples *et al*., 1998; Gong *et al*., 2002; Jorritisma *et al*., 2003, Van Knegsel *et al*., 2005, 2007a, b, c, d; Gilmore *et al*., 2011; Thatcher *et al*., 2011). Evidence has been presented that the improvement in DMI is the critical factor in dairy cows to meet the energy needs for greater amounts of milk produced in early lactation without a more prolonged period of NEB (McGuire *et al*., 2004). In addition, increasing levels of glucogenic or lipogenic dietary components in a diet of dairy cows change plasma energy biomarkers to reduce adverse metabolic and infectious disorders and improve milk synthesis and reproductive function. Lipogenic ingredients, that stimulate the production of butyrate and acetate in the rumen, are expected to increase the ratio of plasma lipogenic/glucogenic compounds (Van Knegsel *et al*., 2005). In addition, feeding dietary fat results in increased energy partition into milk and consequently limits the energy partition into body reserves (Van Knegsel *et al*., 2007a). In contrast, glucogenic nutrients (grain, non-fibre carbohydrates or propylene glycol) are either fermented in the rumen to stimulate the production of propionate or they bypass the rumen and are absorbed in the small intestine as glucose (McDonald *et al*., 2002). Consequently, glucogenic nutrients can increase insulin and glucose levels, thus decreasing the ratio of plasma lipogenic/glucogenic compounds (Van Knegsel *et al*., 2005). As a result of improved insulin and glucose levels, dietary starch stimulates body fat deposition and energy partitioning into body tissue (Van Knegsel *et al*., 2007a). When dairy cows are fed in excess of their daily nutritional requirements, as milk production begins to decline in the final third of the lactating period, they regain a positive EB. At this time, the recovery allows them to replenish body stores through lipogenesis (Bauman & Currie, 1980), in anticipation of the next lactation (Friggens, 2003). Therefore, high insulin levels released from the pancreas activates the enzyme acetyl-CoA carboxylase that promotes fat storage (Drackley, 2000).

2.5. Effect of energy sources on metabolism of dairy cows

Cereal grains, like maize, are primarily fed to provide energy to dairy cows and most of the digestible energy in cereals grains comes from starch (Ali *et al*., 2012). Levels of starch can range up to 30% on a DM basis of the diet in lactating dairy cows (Akins *et al*. 2014). Most of the starch is hydrolysed by various routes to pyruvic acid, which is then fermented in the rumen. This process provides fermentable energy as VFA that is absorbed through the rumen wall (McDonald *et al*., 2002) and enhances the protein/energy balance, as well as rumen MPS (Rearte & Pieroni, 2001; Bargo *et al*., 2003). The rest of the starch, bypassing rumen fermentation, is digested by pancreatic enzymes and absorbed in the small intestine as glucose (Norberg *et al*., 2007). In dairy cows,

dietary starch is efficient to alleviate the NEB, suggesting a reduced postpartum risk of metabolic disorders (Van Knegsel *et al*., 2007c). However, feeding high levels of starch can increase the risk of ruminal acidosis, diminish ruminal fibre digestibility, reduce the acetate/propionate ratio, and alter the composition and level of milk fat (Bargo *et al*., 2003).

Dietary fat improves the energy density of the diet and alters milk fat composition (Schroeder *et al*., 2004). It is almost entirely hydrolysed into fatty acids and hydrogenated in the rumen and later absorbed from the small intestine (Doreau & Ferlay, 1994). Adding more than 8-9% of fat to the diet may result in milk fat and/or milk protein depression due to its negative effect on DMI and rumen fermentation of, especially, fibre (Schroeder *et al*., 2004). To overcome these complications and to improve the energy intake, interest has increased in feeding ruminally inert fats, like Ca-salts of long-chain fatty acids, to lactating dairy cows (Schneider *et al*., 1988). The Ca-salts of long-chain fatty acids are energetically dense and consist of about 51.6% palmitic acid, 5.9% stearic acid, 35.4% oleic acid and 6.2% linoleic acid (Schneider *et al*., 1988). These fats are insoluble at normal ruminal pH and thus inert in the rumen with no effect on microbial fermentation (Chalupa *et al*., 1986). In the abomasum, the fats are broken down by hydrochloric acid to free fatty acids and Ca-ions. The rumen bypass of these fats consequently increases their absorption from the small intestine, potentially enhancing the supply of polyunsaturated fatty acids to the mammary gland (Purushothaman *et al*., 2008). Such milk with modified fat composition has been associated with decreased risk of chronic diseases, including heart disease (Lock & Bauman, 2004).

The inclusions of dietary starch and/or fat have been demonstrated as an effective way to reduce the extent and duration of NEB during early lactation (Williams & Stanko, 2000; Van Knegsel *et al*., 2007c; Garnsworthy *et al*., 2009). As nutrients are digested and absorbed through the GIT, a number of metabolic and hormonal signals released from the liver, pancreas, muscle and adipose tissues act on brain centres, regulating the DMI, the EB and the metabolism of dairy cows (Chagas *et al*., 2007). The signals, which can include amongst others glucose, fatty acids, insulin, IGF-I, insulin, glucagon, GH, ghrelin, leptin and perhaps myostatin, trigger their respective specific receptors by means of positive and/or negative endocrinal feedback mechanisms to regulate DMI, body growth and/or reserves, milk secretion and the reproductive axis (Chagas *et al*., 2007; Lucy, 2007; Garnsworthy *et al*., 2008a; Roche *et al*., 2009; Wathes, 2012; Esposito *et al*., 2014). Furthermore, the latter at the ovarian level is regulated by the hormones of the hypothalamus (gonadotrophin-releasing hormone; GnRH), anterior pituitary (follicle-stimulating hormone; FSH and LH), ovaries (P_4, oestradiol and inhibins) and the uterus (prostaglandin-$F_{2\alpha}$; $PGF_{2\alpha}$) through a

system of positive and/or negative feedback signals to govern the oestrous cycle in dairy cows (Forde *et al.*, 2011). The ovarian follicular growth and development in dairy cows are characterised by three and two consecutive follicular waves in dairy cows and heifers per oestrous cycle, respectively. Each wave begins with the recruitment of a cohort of follicles from the established fixed number of primordial follicles during foetal development and finishes with the selection of a dominant follicle (Webb *et al*., 2004). While other recruited follicles undergo atresia, the dominant follicle continues to grow and mature to the preovulatory stage and eventually ovulates.When cows are in a NEB condition, NEFA and BHB are released from body reserves and used as an alternative energy fuel for other tissues (Vernon, 2002; Esposito *et al*., 2014; Schulz *et al*., 2014). Secondly, the somatotropic axis (consisting of GH, the GH receptor and IGF-I) becomes uncoupled in the liver (Thatcher *et al*., 2010). Thirdly, less ghrelin from the abomasum and more GH from the anterior pituitary gland are released (Chagas *et al*., 2007). Furthermore, less insulin, IGF-I and leptin are released respectively from the pancreas, liver and adipose tissue (Leroy *et al*., 2008b). Lastly, these altered endocrine signals further attenuate the LH pulse frequency and decrease the production of GnRH (Butler, 2003) and therefore supress all together the reproductive axis (Chagas *et al*., 2007). Such metabolic and hormonal depressions, as dictated by the degree and duration of the NEB, influence the ovarian function negatively in terms of the number of follicles, the rate of follicular growth and development, the size of the ovulatory follicle as well as the quality and the viability of the oocyte (Lucy *et al*., 1991; Boland *et al*., 2001; Butler, 2003; Diskin *et al*., 2003; Lucy, 2003; Webb *et al*., 2004; Garnsworthy *et al*., 2008a). In contrast, improvements in these feedback-regulated metabolites (e.g. glucose, amino acids, fatty acids) and hormones (e.g. insulin, IGF-I and leptin) regulate the hypothalamic-pituitary-ovarian-uterine axis positively to enhance fertility outcomes of dairy cows (Leroy *et al*., 2008a, b, c).

Feeding diets that are designed to increase insulin levels during early lactation may increase the proportion of cows ovulating before 50 days postpartum (Gong *et al*., 2002; Van Knegsel *et al*., 2005). Dietary starch inclusion in a diet of dairy cows can improve insulin and glucose levels (Lammoglia *et al*., 1997) and reduce NEFA and BHB levels during the NEB period (Van Knegsel *et al*., 2007b) to eventually promote the resumption of the oestrous cycle (Gong *et al*., 2002; Garnsworthy *et al*., 2008b). However, high starch diets may suppress the appetite and thus the DMI by inducing satiety and shorter meals (Thatcher *et al*., 2011). Furthermore, excessive insulin and IGF-I levels from high starch diets may over-stimulate the ovary to negatively affect the developmental competence of oocytes (Leroy *et al*., 2008c). This over-stimulation results in the production of inferior oocytes due to uncoupled transcriptional factors (i.e. maternal messenger

RNA and protein molecules) in the dominant follicle to acquire the full competence before ovulation (Armstrong *et al*., 2001). The poor transcription of these factors significantly reduced the quality and the viability of the oocyte and decreased after fertilisation the survival of the embryo prior to the embryonic genome activation, occuring at the 8-16 cell stage (Leroy *et al*., 2008b, c). In contrast, the inclusion of dietary fat in a diet of dairy cows enhances the diet energy density stimulating milk production, but also yields higher NEFA and BHB levels associated with lower glucose and insulin levels (McNamara *et al*., 2003; Van Knegsel *et al*., 2005, 2007b; Moallem *et al*., 2007). Furthermore, feeding dietary fat increases the number and size of follicles, as well as the oestradiol production of the preovulatory follicle (Lucy *et al*. 1991; Beam & Butler, 1997; Moallem *et al*., 2007), most likely via the induction of high cholesterol levels in follicular fluid and plasma (Van Knegsel *et al*., 2007a). Vasconcelos *et al*. (2001) reported that an increased follicle size can have advantageous effects on both oocyte quality and corpus luteum function. The resulting high plasma cholesterol concentration also improves $PGF_{2\alpha}$ and P_4 secretion (Staples *et al*., 1998; Staples & Thatcher, 2005; Leroy *et al*., 2014), thus supporting embryo development and pregnancy survival (Ryan *et al*., 1992; Lammoglia *et al*., 1996; McNamara *et al*., 2003).

In summary, optimising the EB status by means of manipulation of dietary starch and/or fat level in diets can potentially be a key tool to enhance the reproductive performance of dairy cows, whilst sustaining milk production. However, a number of hormonal and metabolic signals are involved for a successful reproduction, making physiological pathways with many inter-related factors very complex (Chagas *et al*., 2007; Garnsworthy *et al*., 2008a).

2.6. Effect of energy sources on milk yield and milk composition

Increasing fat- and/or starch-based ingredients into the daily diet increased the milk production of dairy cows (Van Knegsel *et al*., 2005; Reis *et al*., 2012; Higgs *et al*., 2013; Roche *et al*., 2013). A possible explanation for the improved milk production can be attributed to the amount of energy intake, increasing the ME intake with both starch and/or fat ingredients (Bargo *et al*., 2003; Hills *et al*., 2015). Such an enhancement in ME intake was reported to affect the lactation persistence thereafter positively (Hermansen, 1990; Reis *et al*., 2012). Supporting this response, previous studies also reported enhanced milk production as a result of increased energy intake (Erickson *et al*., 1992; Chouinard *et al*., 1997; Moallem *et al*., 2000). However, other studies reported no effect on milk yield when feeding either enriched starch- or fat-based diets or their combinations (Garnsworthy *et al*., 2008b, c, 2009; Gilmore *et al*., 2011; Little *et al*., 2016). These researchers

suggested that the lack of a significant effect on milk production could be attributed to the use of isocaloric diets in the studies.

Milk lactose percentage of dairy cows increased with the inclusion of dietary starch, but decreased with the addition of dietary fat (Van Knegsel *et al*., 2007c). However, other studies reported no effect on milk lactose percentage when either starch or fat were added to diets of dairy cows (Van Knegsel *et al*., 2007a; Garnsworthy *et al*., 2008b, c, 2009). The reason for these differences may be related to either a limited capacity of the mammary gland to absorb increased glucose from the blood or low plasma glucose available for lactose synthesis during early lactation (Piccioli-Cappelli *et al*., 2014). Milk protein percentage of dairy cows decreased with lipogenic diets (Erickson *et al*., 1992; Harrison *et al*., 1995; Chouinard *et al*., 1997). However, glucogenic diets increased the milk protein percentage of dairy cows (Voigt *et al*., 2003), which may be attributed to greater plasma insulin levels (McGuire *et al*., 1995; Van Knegsel *et al*., 2007b), an enhanced MPS in the rumen (Carmo *et al*., 2015) and a greater mammary protein synthesis (Hills *et al*., 2015). Milk fat percentage was usually enhanced after feeding lipogenic diets, but it decreased when feeding glucogenic diets to dairy cows (Van Knegsel *et al*., 2007a, b; Garnsworthy *et al*., 2008b, c; Reis *et al*., 2012). However, overfeeding either dietary starch or fat can lead to a depression in milk fat yield. Van Knegsel *et al*. (2007b) reported that an increase in insulin levels, induced by increased propionate from rumen digestion of starch, can promote gluconeogenesis over lipogenesis due to low availability of fat precursors, to subsequently reduce the fat synthesis in the udder and milk energy output. Another report argued that the depression in milk fat content is possibly caused by an accumulation of trans fatty acids in the rumen because of the low pH with high starch diets (Kalscheur *et al*., 1997). Bauman and Griinari (2001) found that the decrease in milk fat content when overfeeding fat, is generally attributed to altered rumen function, fat biohydrogenation and ruminal formation of trans-10 C18:1 fatty acids. Gama *et al*. (2008) pointed out that an increased supply of trans-10 cis-12 conjugated linoleic acid over other fatty acids to the udder was responsible for milk fat depression in dairy cows. This fatty acid has been recognized as a possible inhibitor of milk fat synthesis, decreasing the activity of lipogenic enzymes in the mammary gland (Baumgard *et al*., 2002).

2.7. Effect of energy sources on reproduction efficiency of dairy cows

Successful reproduction in dairy cows is the consequence of a chain of events after calving, which consists of the resumption of postpartum oestrous function, development and ovulation of a viable oocyte, conception, embryo development, implantation in the uterus, maintenance of pregnancy and

eventually calving (Garnsworthy *et al*., 2008a). A failure at any of these steps results in the failure of a successful conception and embryonic/pregnancy survival (Leroy *et al*., 2008a, b). Because of this, the fertility of dairy cows is defined as a multi-factorial trait (Butler, 2003). The general decline in fertility has been attributed to a network of genetic, environmental and managerial factors and their interactions, making it difficult to determine the exact reason for the deterioration in cow fertility (Walsh *et al*., 2011). So for example, a decline in the fertility of dairy cows has been attributed to the reduced ability of the uterus to recover after calving, longer anovulatory periods and behavioural anoestrus, poor oestrous signs, irregular oestrous cyclicity, poor oocyte quality, poor fertilisation, abnormal embryonic implantation and/or foetus development, uterine/placental incompetence and pregnancy loss (Mwaanga & Janowski, 2000; Lucy, 2007; Wathes *et al*., 2007; Leroy *et al*., 2008c; Evans & Walsh, 2012).

Endocrine status, interval from calving to first oestrus, conception rate and pregnancy maintenance are all altered when reduced DMI and/or longer periods of NEB are manifested in cows (Mwaanga & Janowski, 2000). Increasing the amount of dietary starch and/or fat in the diet reduced the interval from calving to first ovulation and therefore initiated earlier postpartum cyclicity in cattle (Lammoglia *et al*., 1996; Gong *et al*., 2002; Santos *et al*., 2008; Burke *et al*., 2010). The later evident response can be attributed to the improved EB status as the somatotropic axis synergise with the gonadotropins on ovarian cells, allowing the dominant follicle to ovulate and resuming the oestrous cycles thereafter. However, other studies reported no or negative effects of the energy intake level on the number of days from calving until the first oestrus (Beam & Butler, 1997, 1998; Garcia-Bojalil *et al*., 1998; Oldick *et al*., 1997; Garnsworthy *et al*., 2009). Gong *et al*. (2002) reported increased conception rates following the first insemination when feeding dietary starch. In contrast, other investigations found no or negative effects of the energy intake level on the conception rate following the first insemination (McNamara *et al*., 2003; Garnsworthy *et al*., 2009, Gilmore *et al*., 2011). Furthermore, some studies found improved pregnancy rates when feeding either dietary starch or fat to dairy cows (Burke *et al*., 2010; Reis *et al*., 2012), while others reported no or negative effects (McNamara *et al*., 2003; Dyck *et al*., 2011; Gilmore *et al*., 2011). However, important enhancements in conception rate were observed when feeding a diet that increased glucose and insulin levels in the early postpartum period and then switching to a diet that reduced insulin levels during the mating period, compared to other treatments (Garnsworthy *et al*., 2009). Furthermore, pregnancy rates to first and second services were enhanced when grass silage was supplemented with a similar concentrate fed to cows individually based on milk yield of the previous week, compared to those on a mixed diet containing grass silage and concentrate on a

50:50 ratio on a DM basis (Little *et al.*, 2016). In contrast to this report, Gilmore *et al*. (2011) found no improvements in pregnancy rates when feeding a glucogenic diet in early lactation to encourage the resumption of oestrus followed by a lipogenic diet to promote embryonic development, compared to other treatments. These researchers suggested that the lack of significance was due to the small number of animals used in the study.

Several causes can potentially contribute to the inconsistency in effects of dietary starch and/or fat on reproduction performance of dairy cattle in previous studies. Firstly, the levels and types of dietary fat (chain length and degree of saturation of long chain fatty acids) and starch (rate of fermentation in the rumen) have directly affected the profile of nutrients absorbed through the GIT and indirectly acted on the EB status, which both in return probably influenced the ability to conceive and remain pregnant (Staples *et al*., 1998; Van Knegsel *et al*., 2007a; Leroy *et al*., 2008c; Roche *et al*., 2011). Secondly, it is critical to distinguish between non-isocaloric and isocaloric diets in studies, since the energy density, defined by the nutrient content (starch *vs* fat), has been described to have significant effects on reproduction efficiency (Van Knegsel *et al*., 2005). Another source of variation could be the difference in number of animals, protocols and interpretations of experimental results between studies (McNamara *et al*., 2003; Gilmore *et al*., 2011).

Usually, feeding dietary starch that promotes glucose and insulin levels (Garnsworthy *et al*., 2008b) favours an early resumption of the first postpartum ovulation (Gong *et al*., 2002), while decreasing the quality of oocytes (Armstrong *et al*., 2001) and the conception rate thereafter (Leroy *et al*., 2008b). Plasma NEFA and BHB levels are increased and insulin levels are decreased with dietary fat inclusion (Leroy *et al*., 2008c), resulting in a longer anoestrous period (Giuliodori *et al*., 2011). However, dietary fat improves the quality of oocytes and corpus luteum (Beam & Butler; 1997; Vasconcelos *et al*., 2001), while increasing the P_4 levels to enhance the pregnancy success (Roche *et al*., 2011). Both observations support the possible existence of nutritional signals associated with dietary energy sources, dependently or independently of EB, that influence the reproduction axis through signals to the ovarian, oviductal and uterine organs (Wathes *et al*., 2007). Despite all the progress made in this field, the physiological pathways explaining the link between EB indicators, hormonal and metabolic signals and their specific receptors, and pregnancy success remain, to a certain extent, unclear (Chagas *et al*., 2007). Also, feeding trials that investigated the effect of energy levels and sources from calving to mid- or late lactation on reproductive performances are limited, thus making it difficult to draw final conclusions.

2.8. Conclusion

Inclusion levels and types of dietary energy sources, such as starch and/or fat, affect plasma metabolite profiles, milk production and fertility of dairy cows. Nutritional management before and after calving must facilitate a successful metabolic adaptation in the liver and rapid increases of postpartum DMI, indispensable for improved milk production and efficient reproductive performance. This review demonstrated that there are definite physiological and metabolic links in dairy cows between the amounts and types of dietary energy nutrients absorbed through the GIT and biological responses such as metabolic profiles, endocrinal activity, milk secretion and reproduction outcomes. However, fertility before the establishment of oestrus in heifers or the resumption of oestrus in postpartum cows to the next calving is not only complex and multifactorial, but is in the decline worldwide. In addition, bovine results on prepartum and postpartum effects of energy sources and levels on milk production and reproduction are limited. This is an area of research that needs attention and will form the basis of investigations presented in this thesis.

2.9. References

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Chapter 3

Effect of energy sources on blood metabolite profiles and live weight changes as an indication of the nutritional status in Holstein cows

Abstract

The objective of the study was to evaluate the effect of nutritional treatments differing after calving in energy levels and sources on plasma metabolite profiles and live weight (LW) changes as an indication of the nutritional status in Holstein cows. During the dry period (DP), pregnant heifers (n=69) and dry cows (n=153) from the Elsenburg Research Farm were maintained under similar feeding and management conditions. Following parturition, cows had *ad libitum* access to cultivated irrigated kikuyu-ryegrass pastures and were assigned to three groups according to calving date, parity, LW and milk yields of their previous lactation. The groups were supplemented with different levels and types of concentrates of which the energy was provided by starch and/or fat. The control group was offered 7 kg/day of a control concentrate supplement for both primiparous and multiparous cows, while concentrates in treatment groups were fed at 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively. The concentrate components of the respective treatments consisted of high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) combination, respectively. The HSLF supplement was a glucogenic concentrate containing maize as the energy source. The HSLF-LSHF supplements consisted of a glucogenic concentrate offered for the first 60 days in milk (DIM) as per treatment HSLF, and followed from 61 DIM by a lipogenic concentrate containing wheat bran and calcium (Ca)-salts of long-chain fatty acids as the energy sources. Results showed that all cows mobilised their body fat reserves as evidenced in changes in plasma non-esterified fatty acids (NEFA) before and after calving. Postpartum plasma NEFA and β-hydroxybutyrate (BHB) did not differ significantly between nutritional treatments in multiparous cows. However, the postpartum levels of plasma NEFA and BHB were higher ($P < 0.05$) for the control, indicating a status of advanced negative energy balance (NEB) and possible subclinical ketosis, compared to HSLF and HSLF-LSHF treatments in primiparous cows. Postpartum plasma urea levels decreased ($P < 0.05$) in both primiparous and multiparous animals in the control group, in comparison to the HSLF and HSLF-LSHF groups. As affected by time, the postpartum LW was the lowest ($P < 0.05$) and the LW loss was the highest ($P < 0.05$) in cows receiving the control supplements in comparison to HSLF and HSLF-LSHF supplements for both primiparous and multiparous cows. In addition, the LW $loss_{nadir}$ and the number of days to reach LW_{nadir} increased ($P < 0.05$) in primiparous cows receiving the control

concentrate, compared to those of the HSLF and HSLF-HFLS treatments. However, this trend was not observed for multiparous cows. Findings of this study showed that HSLF and HSLF-LSHF treatments improved the nutritional status, as was evident in a reduced extent of NEB and limited LW loss of dairy cows, compared to those in a control group.

3.1. Introduction

During the transition period, dry matter intake (DMI) does not generally satisfy the increasing nutrient demand of dairy cows, mainly because of a decrease in feed intake and appetite (Walsh *et al*., 2011). As a result, dairy cows go into a state of NEB and thus mobilise body reserves as a physiological mechanism to adapt to the energy deficiency from late gestation to early lactation (Rukkwamsuk *et al*., 1999; Van Knegsel *et al*., 2007b; McArt *et al*., 2013). The NEB starts a few days before parturition, reaching a NEB nadir 2 to 4 weeks postpartum, with the energy balance (EB) becoming positive again by 10 to 12 weeks after calving (Butler, 2003). During the NEB period, NEFA are intensively released from adipose tissues and recycled as energy fuels (Adewiyi *et al*., 2005; Hammon *et al*., 2009). Such strong mobilisation of body reserves induces imbalances in fat and carbohydrate metabolism with an increase in ketone bodies and tri-acyl glycerol (TAG) respectively, leading to sub- or clinical ketosis (Schulz *et al*., 2014) and fatty liver syndrome (Drackley *et al*., 2001).

A state of NEB results in a loss of body score condition (BCS) and LW (Jorritsma *et al*., 2003; Van Straten *et al*., 2008). Measurement of LW is an established method for monitoring the growth performance of livestock such as beef cattle, sheep and broilers. Using LW as a tool to assess EB in dairy cows has however been discouraged due to the possible confounding effects of parity, stage of lactation, frame size, stage of gestation, rumen fill and breed (Alawneh *et al.*, 2012; Thorup *et al*., 2012). Roche *et al*. (2009) reported that measurement of postpartum LW does not strictly offer a true reflection of the EB status of an individual cow. This report was correct as LW of cows in milk were mainly being measured on a cross-sectional basis (i.e. at certain key points in the dairy production cycle e.g. milk tests) in many studies. However, the use of continuous LW monitoring on a daily or weekly basis can possibly deliver efficient means to monitor on-farm management-related performances in dairy herds (Sakaguchi, 2009). This technique could potentially estimate EB status in identifying cows that continue to lose or gain weight over time (Jorritsma *et al*., 2003; Thorup *et al*., 2013). Of interest, LW is an easy and fast method to use at the farm level, compared to the inherent difficulties with BCS measurement due to inter-observer inconsistency and bias (Broster & Broster, 1998; Van Straten *et al*., 2008). Also, the continuous walkover or walk-through

weighing system installed at the exit of the milking parlour is non-intrusive and a relatively accurate tool that has the capacity to reduce stress on animals.

Numerous nutritional approaches to improve the plasma metabolite profiles and prevent the LW loss have been investigated. Some studies demonstrated that enhancing the postpartum metabolizable energy (ME) intake, by either reducing the forage to concentrate ratio or by supplementing energy-dense ingredients like dietary starch and/or fat, reduces the extent and the duration of the NEB and improves the LW of dairy cows (Drackley *et al*., 2003; Staples *et al*., 1998; Voigt *et al*., 2003; Van Knegsel *et al*., 2005; Gilmore *et al*., 2011). The objective of the current study was therefore to investigate the effect of nutritional treatments differing after calving in energy levels (low *vs* high) and sources (starch *vs* fat) on blood metabolite profiles and LW changes until 154 days postpartum, and to determine the extent to which these parameters can provide an indication of the postpartum nutritional status and health of Holstein dairy cows.

3.2. Materials and Methods

The ethical clearance for this study was obtained from the Western Cape Department of Agriculture (WCDA, Project AP/BR/D/CM31).

3.2.1. Experimental location

The study was conducted at the Elsenburg Research Farm of WCDA located approximately 50 km east of Cape Town at an altitude of 177 m, longitude of 18° 50' and latitude of 33° 51' in the winter rainfall region of South Africa. The area receives an average annual rainfall of 650 mm and has a typical Mediterranean climate with short, cool, wet winters and long, warm, dry summers.

3.2.2. Experimental husbandry, animals and diets

Holstein cows from the Elsenburg herd that had calved from August 2012 to July 2014 were used in the study. Cows were included in the study from 4 weeks before parturition to 22 weeks postpartum. Fresh drinking water was freely available at all times in the trial. During the DP of the trial, pregnant heifers and dry cows were maintained in two separated camps on the same feeding and management conditions. Animals were fed unchopped oat hay *ad libitum* supplemented with a prepartum concentrate mixture, containing maize as the main energy source and anionic salts, at 3 kg/day for heifers and cows from 30 to 14 days prepartum, and thereafter from 13 days prepartum to the calving date, 6 and 5 kg/day for cows and heifers, respectively. Following parturition, cows were allocated into three nutritional treatments, based on parity, LW at calving and/or milk yields during the previous lactation period. Additionally, allocations to nutritional programmes were

strictly done in both parity groups, according to calving dates, so that animals were exposed to similar environment conditions during the observation period of the trial. Primiparous animals were assigned based on their LW and calving dates. Live weights of primiparous cows at calving were 502 ± 14, 493 ± 13 and 491 ± 9 kg for the control, HSLF and HSLF-LSHF groups respectively. Primiparous cows were 2.22 ± 0.05, 2.18 ± 0.04 and 2.27 ± 0.05 years of age at calving for the control, HSLF and HSLF-LSHF groups, respectively. Multiparous animals were assigned according to parity, previous lactation milk yield and calving dates. Live weights of multiparous cows at calving were respectively 621 ± 11, 630 ± 10 and 620 ± 11 kg for the control, HSLF and HSLF-LSHF groups. Multiparous cows were 5.35 ± 0.20, 5.11 ± 0.29 and 5.15 ± 0.26 years of age at calving for the control, HSLF and HSLF-LSHF groups, respectively. The milk yields in the previous lactation of multiparous cows were 8880 ± 141, 8869 ± 156 and 8799 ± 180 kg for the control, HSLF and HSLF-LSHF groups, respectively.

During the postpartum period of the trial, cows were offered unrestricted access to cultivated kikuyu-ryegrass pastures and were supplemented with pelleted concentrates. Pastures were irrigated, as required, using a permanent irrigation system. Cows followed a rotation programme to ensure an *ad libitum* DMI under normal conditions. A partial total mixed ration (pTMR) consisting of oat hay (48%), lucerne hay (43%) and soybean oil cake meal (9%) was provided *ad libitum* as additional roughage when pasture availability was low. Due to the experimental design, it was not practical to determine individual or group feed intake between treatments, because animals were allowed to graze as one herd on pastures for the duration of the experiment. Cows were milked twice daily at 05:30 and 15:00. Cows received half of the daily concentrate after each milk session in the milking parlour. Concentrates in treatments differed in energy levels (low *vs* high) and sources (starch *vs* fat).

The control was the standard pasture feeding system with an allowance of a control concentrate of 7 kg/day from calving until 154 DIM for both primiparous and multiparous dairy cows. The supplement in treatment HSLF was offered from calving to 154 DIM and was a glucogenic concentrate containing high starch-low fat levels, using maize as the energy source. The supplements in treatment HSLF-LSHF were combinations of a glucogenic concentrate, offered for the first 60 DIM as per treatment HSLF and followed from 61 to 154 DIM by a lipogenic concentrate with low starch-high fat levels containing wheat bran and Ca-salts of long-chain fatty acids (Megalac rumen bypass fat, Volac International Ltd., UK) as the energy sources. The

concentrate allowances in treatments HSLF and HSLF-LSHF were 11.6 kg/day for primiparous and 12.6 kg/day for multiparous dairy cows, respectively.

Representative samples of feeds were collected weekly, then bulked monthly and analysed for chemical composition. The AOAC (1990) official methods were used to determine dry matter (DM), ash, fat and crude protein (CP) (Nitrogen, Nx6.25) contents of feedstuffs and pastures. Neutral detergent fibre (NDF) was determined according to Van Soest *et al.* (1991), with the use of sodium sulphite anhydrous and amylase to decrease contaminations of nitrogen and starch in the NDF determination. Calcium and P were determined using an inductively coupled plasma dry ashing method (ALASA, dry ashing 6.1.1, RevA/98) with an Iris Advantage Thermo elemental instrument according to Giron (1973). Table 3.1 sets out the chemical composition of feeds used in the trial. Figure 3.1 represents the pasture quality parameters over the duration of the study.

Table 3.1 The chemical composition of feeds for dairy cows used in the study.

	Concentrates						
Parameters	**Control**	**HSLF[2]**	**LSHF[2]**	**Dry period**	**Pasture[2]**	**pTMR[2]**	**Oat hay**
DM[1] (g/kg as is)	894	899	888	891	210	917	932
Ash[1] (g/kg DM)	72	65	75	106	103	69	41
CP[1] (g/kg DM)	179	176	182	188	184	152	48
Fat as EE Total[1] (g/kg DM)	42	35	87	36	34	19	16
NDF[1] (g/kg DM)	200	99	197	179	543	500	689
Ca[1] (g/kg DM)	14	12	13	29	5	5	3
P[1] (g/kg DM)	7	9	10	7	5	3	2

[1]Determined from laboratory analysis of components, with DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fibre, Ca: Calcium and P: Phosphor.

[2]With HSLF: High starch-low fat, LSHF: Low starch-high fat, Pasture: Irrigated kikuyu-ryegrass pastures and pTMR: Partial total mixed ration.

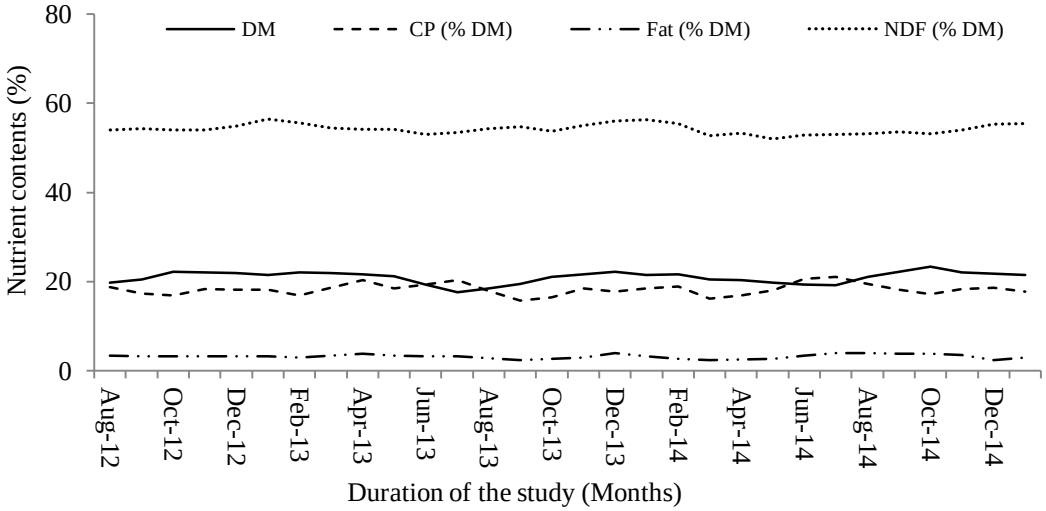

Figure 3.1 A graphic representation of pasture quality parameters monitored over the duration of the study. Values were depicted on a DM basis.

Table 3.2 Determined composition of the experimental control, HSLF and LSHF diets.

Postpartum diets[4]	Control	HSLF[3] Treatment	LSHF[3] Treatment
Physical composition			
Ryegrass-Kikuyu pastures (%)	75.0	54.7	55.3
Control concentrate (%)	25.0	-	-
HSLF[3] concentrate (%)	-	45.3	-
LSHF[3] concentrate (%)	-	-	44.7
Chemical composition			
DM[1] (%)	38.1	52.2	51.3
ME[2] (Mcal/kg)	2.47	2.71	2.70
NE_L[2] (Mcal/kg)	1.59	1.74	1.74
NDF[1] (g/kg DM)	457	341	388
NFC[2] (g/kg DM)	255	374	289
Starch[2] (g/kg DM)	104	242	137
Fat as EE Total[1] (g/kg DM)	40	35	58
CP[1] (g/kg DM)	183	180	183
RDP[2] (% CP)	62	63	63
Ash[1] (g/kg DM)	96	86	91
Ca[1] (g/kg DM)	7.1	8.1	8.4
P[1] (g/kg DM)	5.1	6.3	6.8

[1]Determined from chemical composition of feeds (see Table 3.1), with DM: Dry matter, NDF: Neutral detergent fibre, EE: Ether extract, CP: Crude protein, Ca: Calcium and P: Phosphor.

[2]Calculated from feed formulation package (CPM-Dairy, 2006) using multiparous cows of 590 kg of Live weight, with ME: Metabolizable energy, NE_L: Net energy lactation, NFC: Non-fibre carbohydrate and RDP: Rumen degradable protein.

[3]HSLF: High starch-low fat and LSHF: Low starch-high fat.

[4]Diets were estimated on DM basis using an intake of 22 and 25 kg/day for primiparous and multiparous cows, respectively.

Using the CPM-Dairy software programme (2006), postpartum diets as presented in Table 3.2 were formulated on DM basis using an intake level of 22 and 25 kg DM/day for primiparous and multiparous cows, respectively. Diets consisted of the different levels and types of concentrates offered between treatments and the rest of the intake being supplied by pastures. The postpartum diets were formulated to be isonitrogenous. The control diet contained a low energy level of 2.47 Mcal ME/kg DM and had 457 g/kg of total NDF on DM basis. The HSLF and LSHF contained high levels of energy of 2.71 and 2.70 Mcal ME/kg DM, respectively and were both formulated to improve metabolic and hormonal responses that benefit the restoration of postpartum LW. The HSLF diet consisted of 242 g/kg of total starch content on DM basis. This HSLF diet was fed to increase plasma insulin and glucose levels in order to improve the EB status. In the HSLF-LSHF combination treatment, the high starch-based diet was initially fed during the first 60 DIM to achieve the same objective of the HSLF treatment. Lastly, LSHF diet was fed from 61 DIM to

improve the EB status using a rumen bypass fat at an inclusion level of 58 g/kg on DM basis, while decreasing plasma insulin level and improving plasma cholesterol level.

3.2.3. Experimental sampling and data collection

During the prepartum period, pregnant heifers and dry cows were weighed once every week until parturition. Calving seasons were summer from 15 October to 14 April with December to February being the hottest months and winter from 15 April to 14 October with June to August being the coldest months. Postpartum LW of dairy cows was automatically recorded daily after milking sessions using a calibrated electronic scale. Daily LW data were re-calculated to weekly means. The nadir point was defined as the DIM in which the lowest postpartum LW value was recorded (Sakaguchi, 2009). The weekly LW loss was estimated as follows:

$LW\ loss_n = (LW_{week1} - LW_n) \times 100(\%) / LW_{week1}$

Where $LW\ loss_n$ and LW_n were respectively LW loss and LW at *n* weeks after parturition

LW_{week1} was LW within one week after parturition.

The rate of LW loss from calving to LW_{nadir} was estimated in kg/day as follows:

Rate of LW loss from calving to $LW_{nadir} = (LW_{at\ calving} - LW_{nadir}) /$ Number of days to reach LW_{nadir}.

The rate of LW gain from LW_{nadir} to 154 DIM was estimated in kg/day as follows:

Rate of LW gain from LW_{nadir} to 154 DIM $= (LW_{at\ 154\ DIM} - LW_{nadir}) / (154 -$ Number of days to reach LW_{nadir}).

Records on prepartum and postpartum LW changes of 222 primiparous and multiparous Holstein cows were available for analyses. Among these cows, 84 were blocked, based on calving date and parity, and monitored for plasma metabolites. Bloods were sampled in the summer season form October 2013 to June 2014. Blood samples were collected from the central middle coccygeal vein on the tail (Sears *et al.*, 1978) every week from 4 weeks prepartum to 4 weeks postpartum and after that, every three weeks up to 13 weeks after calving. Following blood collection at 06h00, samples were centrifuged, cooled after which the serum samples were transported using a cooler box and ice packs to a veterinary laboratory for analyses. Serum samples were analysed for plasma NEFA, BHB and urea using infrared spectrography (Thermo Scientific INDIKO analyser, Finland, 2011) and commercially available kits (NEFA: FA 115 kit, Randox Laboratories Ltd, Crumlin, UK; BHB: Ranbut kit, Randox Laboratories Ltd, Crumlin, UK; and plasma urea: Urea Thermo Scientific INDIKO procedure reference 981818/981820). Plasma concentrations of BHB were only analysed during the postpartum period. The accuracy of each assay was monitored with the use of a

commercial reference serum sample (Bovine precision serum, Randox Ltd) and all coefficients of variation between analyses were < 5%.

3.2.4. Statistical analysis

Data were analysed using the PROC MIXED of SAS enterprise guide (SAS, 2012), grouped according to parity groups i.e. primiparous or multiparous cows. To avoid problems of fitting a covariance structure in the statistical model, prepartum and postpartum data were analysed separately. The statistical model included year (*Y*) effect, calving season (*S*) effect, treatment (*T*) effect, week (*W*) effect of observations and the interaction effects between treatment and week of observation (*WT*) as well as treatment and calving season (*ST*) as fixed effects, while the animal effect within treatments was specified as a random effect. The effects of year and season of calving were included in the statistical model because of the duration of the experiment. The measured variables obtained every day within a particular week during the trial were considered as repeated observations of that particular week block. The approach was to account the daily data variations within each week of observations in order to reduce the error variance of treatment effects in the statistical analysis. Secondly, this repeated observation permitted a longitudinal analysis to monitor how the weekly means changed over time. The statistical model was as follows:

$Model = \mu + Y_i + S_j + T_k + W_l + (ST)_{jk} + (WT)_{kl} + \delta_{(lk)m} + \varepsilon_{ijklm}$, where

μ = overall mean;

Y_i = the fixed effect of the i^{th} year of calving (i.e. 2012, 2013 and 2014);

S_j = the fixed effect of the j^{th} season of calving (i.e. summer and winter);

T_k = the fixed effect of the k^{th} treatment (i.e. Control, HSLF and HSLF-LSHF);

W_l = the fixed effect of the l^{th} week of observation (i.e. 1 to 22)

$(ST)_{jk}$ = the interaction between levels of the j^{th} season of calving and k^{th} treatment;

$(WT)_{kl}$ = the interaction between levels of the l^{th} week of sampling and k^{th} treatment;

$\delta_{(kl)m}$ = the variable effect of the m^{th} block effect in the k^{th} treatment (Repeated statement);

ε_{ijklm} = the random experimental error.

Prepartum LW were analysed without the repeated statement ($\delta_{(kl)m}$) while all effects were used to analyse postpartum LW in the statistical model. Because blood was collected every week in one summer season, plasma metabolites were analysed without the repeated statement ($\delta_{(kl)m}$), as well as the year and calving season effects in the statistical model. The LW traits generated from the postpartum LW curves were analysed without the repeated statement ($\delta_{(kl)m}$) using year, calving season, treatment and the interaction between treatment and calving season as fixed effects in the

statistical model. Statistical assumptions were described as fixed effects and their interactions were equal to zero with $\delta_{(kl)m} \sim N(0, \sigma_e^2)$ varying independently of ε_{ijklm}. Differences in means and standard error (SE) of means between treatments were obtained using the pair wise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

3.3. Results and Discussion

Table 3.3 The effect of nutritional treatments differing after calving in energy levels and sources on the non-esterified fatty acid and β-hydroxybutyrate (mean ± SE) levels recorded for primiparous and multiparous dairy cows during a four week prepartum to 13 week postpartum period.

	Concentrate feeding			P-values		
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[2]**	***W*[2]**	***WT*[2]**
Primiparous cows						
Number of cows	13	6	6			
Non-esterified fatty acid (mmol/L)						
Prepartum	0.29 ± 0.05	0.32 ± 0.06	0.31 ± 0.03	0.34	0.82	NS
Postpartum	0.56[a] ± 0.08	0.37[b] ± 0.06	0.37[b] ± 0.08	0.02	0.12	NS
β-Hydroxybutyrate (mmol/L)						
Postpartum	0.57[a] ± 0.04	0.35[b] ± 0.04	0.43[b]± 0.03	0.001	0.92	NS
Multiparous cows						
Number of cows	25	19	19			
Lactation number	3.82 ± 0.17	3.49 ± 0.22	3.54 ± 0.22			
Non-esterified fatty acid (mmol/L)						
Prepartum	0.25 ± 0.02	0.24 ± 0.02	0.28 ± 0.03	0.19	0.002	NS
Postpartum	0.29 ± 0.02	0.28 ± 0.03	0.30 ± 0.03	0.50	<.001	NS
β-Hydroxybutyrate (mmol/L)						
Postpartum	0.51 ± 0.02	0.47 ± 0.02	0.49± 0.02	0.40	0.31	NS

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat. [2]*T*: Treatment, *W*: Week, *WT*: Interaction WT.

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

The effects of nutritional treatments on the plasma NEFA and BHB for primiparous and multiparous cows are presented in Table 3.3. As expected, plasma NEFA did not differ ($P > 0.05$) during the prepartum period in both the primiparous and multiparous groups, as cows were maintained under the same prepartum feeding and management conditions. Postpartum plasma NEFA and BHB were similar between treatments for multiparous cows. Also, an interaction between treatment and week of observation was not recorded for multiparous cows. In the primiparous cows, postpartum plasma NEFA and BHB increased $(P < 0.05)$ in animals offered the control concentrate, compared to animals receiving HSLF and HSLF-LSHF concentrates. However, the interaction between diet and time of observation was not affected. In both parity groups, the

changes in NEFA and/or BHB over time were as expected during transition (Figures 3.2 and 3.3). These variations in both parity groups indicated the overstressed ability of cows to adapt to the energy shortfall caused by the energy requirement exceeding the nutrient intake around parturition. Supporting the current results of energy metabolites, other studies found that plasma NEFA and BHB levels were significantly elevated in cows during early lactation (Adewiyi *et al*., 2005; Van Knegsel *et al*., 2007a; McArt *et al*., 2013; Schulz *et al*., 2014).

The variations in plasma NEFA and BHB over time are illustrated in Figures 3.2 and 3.4 for primiparous cows and Figures 3.3 and 3.5 for multiparous cows, respectively.

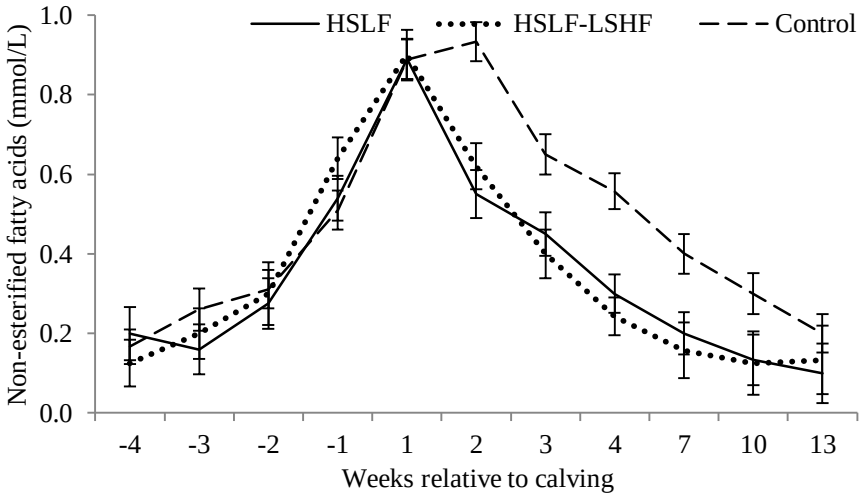

Figure 3.2 The effect of nutritional treatments differing after calving in energy levels and sources on non-esterified fatty acid (mean ± SE) levels recorded for primiparous dairy cows during a four week prepartum to 13 week postpartum period.

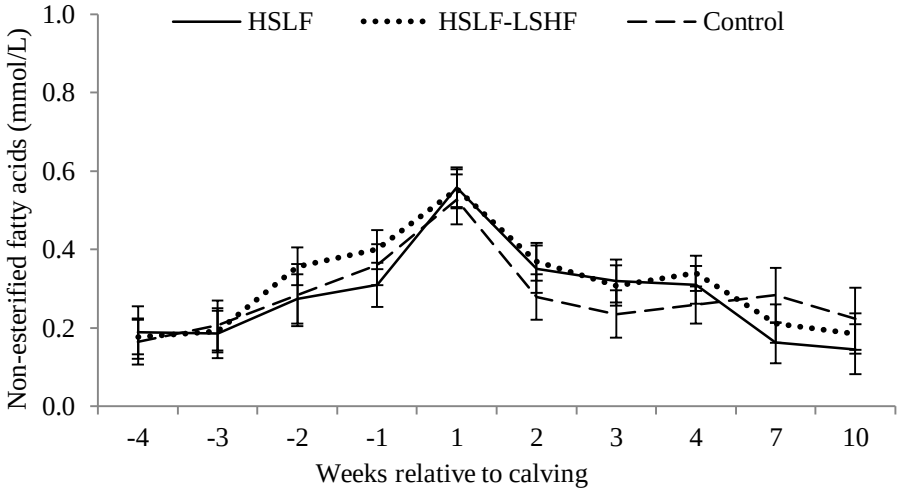

Figure 3.3 The effect of nutritional treatments differing after calving in energy levels and sources on the non-esterified fatty acid (mean ± SE) levels recorded for multiparous dairy cows during a four week prepartum to 13 week postpartum period.

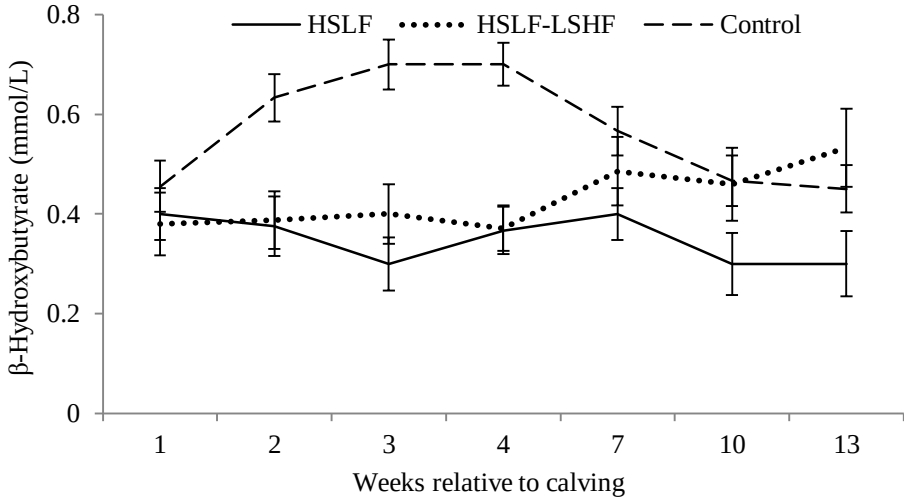

Figure 3.4 The effect of nutritional treatments differing after calving in energy levels and sources on the β-hydroxybutyrate (mean ± SE) levels recorded for primiparous dairy cows during a 13 week postpartum period.

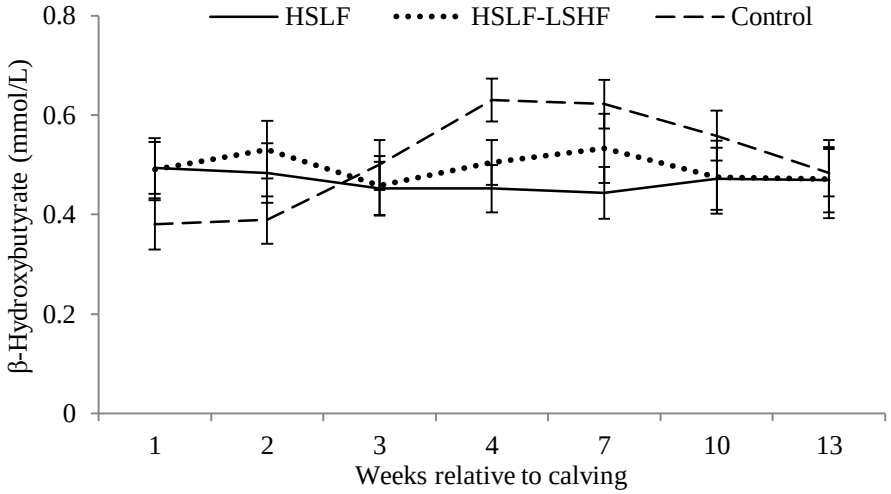

Figure 3.5 The effect of nutritional treatments differing after calving in energy levels and sources on the β-hydroxybutyrate (mean ± SE) levels recorded for multiparous dairy cows during a four week prepartum to 13 week postpartum period.

A NEB generally occurs before and after calving since the demands for glucose, fatty acids and amino acids for foetal growth or milk production outweigh the increase in voluntary DMI in dairy cows (Hammon *et al.*, 2009), due to limitations in intake and appetite (Overton & Waldron, 2004; Walsh *et al.*, 2011). In addition, plasma somatotropin increases after calving, resulting in the partitioning of absorbed nutrients from digestion in the liver and from adipose tissues towards milk production (Chagas *et al.*, 2007). In the liver, the increase in growth hormone (GH) directly stimulates gluconeogenesis and indirectly antagonises the production of insulin, essentially for meeting glucose demands for milk production (Lucy, 2004). In adipose tissue, GH increases

lipolysis. This breakdown supplies fatty acids to the mammary gland for the synthesis of the milk triglycerides (Drackley, 2000; Van Knegsel *et al*., 2007a) and alternative energy sources in the form of NEFA and/or BHB used as additional fuels to meet the energy requirements (Goff & Horst, 1997; Van Knegsel *et al*., 2007a, b). A certain level of NEFA and BHB in the bloodstream is considered in periparturient cows as a normal adaptation to the NEB (McArt *et al*., 2013). Levels of plasma NEFA and/or BHB slowly increase at the end of the DP, as cows approach calving time, and peak at calving (Adewiyi *et al*., 2005). Levels remain high after calving, indicating a status of NEB and/or ketosis (Schulz *et al*., 2014). The levels of plasma NEFA and/or BHB decline to lower levels by six weeks after calving (Adewiyi *et al*., 2005). This reaction occurs as a periparturient physiological mechanism, indispensable for nutrient partitioning for foetal needs, beginning of lactogenesis, stresses of calving and changes in physiological, metabolic and endocrinal status to allow parturition and lactogenesis (Contreras & Sordilla, 2011; McArt *et al*, 2013).

Importantly, feeding glucogenic-based diets during early lactation resulted in decreased plasma NEFA and BHB concentrations (Lemosquet *et al*., 1997; Pickett *et al*., 2003; Reist *et al*., 2003), while lipogenic-based diets led to increased plasma NEFA and BHB (Moallen *et al*., 1997; Drackley *et al*., 2003; Ponter *et al*., 2006). However, other studies showed no effects of energy based diets on energy biomarkers, probably due to the similar energy levels between diets (Van Knegsel *et al*., 2007a; Roche *et al*., 2013). Van Knegsel *et al*. (2007b) found that plasma NEFA and BHB of primiparous cows were similar, but differed in multiparous cows, with levels being significantly higher on cows fed mixed and lipogenic diets, compared to a glucogenic diet. Recently, the association of different cut-points of elevated NEFA and BHB levels with adverse postpartum health and production problems have been investigated in identifying animals with poor adaptation to NEB. Prepartum NEFA cut-points measured 14 to 3 days prior to calving ranged from 0.3 to 0.5 mmol/L, whereas postpartum cut-points measured from 1 to 14 DIM were from 0.70 to 1.0 mmol/L for NEFA and 0.9 to 1.4 mmol/L for BHB, respectively (Ospina *et al*., 2010a, b; Chapinal *et al.*, 2012). Above these cut-points, postpartum disruptions can include increased incidences of peripartum diseases, lower milk yields, early culling from the herd and poor fertility (Van Knegsel *et al*., 2007a; Giuliodori *et al*., 2011; Seifi *et al*., 2011; Evans & Walsh, 2012; McArt *et al*., 2013; Esposito *et al*., 2014). In this study, changes in plasma NEFA over time indicated that all cows relied *($P < 0.05$)* on the mobilisation of body reserves (i.e. NEFA > 0.5 mmol/L in one week before calving and peaking after calving; Figure 3.2 for primiparous and Figure 3.3 for multiparous cows, respectively) to satisfy the nutrient demand during transition. Also, the increase in postpartum plasma NEFA and BHB levels ($P < 0.05$) in grazing primiparous cows receiving 7

kg/cow per day of concentrate in the control group indicated higher lipolysis and possible subclinical ketosis compared to those receiving 11.6 kg/cow per day of concentrates in the HSLF and HSLF-LSHF treatment groups (Figures 3.2 and 3.4). One possible explanation for the alterations in energy biomarkers can be attributed to the high starch and/or fat levels being fed to cows. Although not measured in this study, feeding more starch and/or fat in HSLF and HSLF-LSHF treatments presumably increased glucose and insulin levels (Garnsworthy *et al.*, 2008a; Lammoglia *et al.*, 1997) and subsequently facilitated the uptake of NEFA (Van Knegsel *et al.*, 2007a; Garnsworthy *et al.*, 2008a, 2009; Lee & Hossner, 2002) and decreased lipolysis of body fat reserves (Lafontan *et al.*, 2009), compared to the control.

Another possible explanation can be related to the difference in parity of dairy cows. Normally, dairy heifers usually calved for the first time at approximately 24 months of age, making them not physically mature at this stage (Coffey *et al.*, 2006). Primiparous cows during transition are thus in a different metabolic state compared to multiparous cows as they need nutrients for their own growth in addition to that of the developing calf before calving or milk synthesis after calving (Wathes *et al.*, 2007; McArt *et al.*, 2013). The observed variations in NEFA and BHB in this study suggest possible divergences in the control of mobilization of body reserves between primiparous and multiparous cows, which encourage nutrient partitioning into the animal growth and milk production during the first lactation. The lack of effects of nutritional treatments on postpartum NEFA and BHB levels can be attributed to the possible advanced hepatic adaptation in matured multiparous cows (parity > 3) to postpartum energy stress, compared to young and still growing primiparous cows.

The effects of nutritional treatments on blood urea for primiparous and multiparous cows are presented in Table 3.4. Changes in plasma urea over time are illustrated in Figure 3.6 for primiparous cows and Figure 3.7 for multiparous cows, respectively. As expected, prepartum plasma urea levels did not differ significantly between treatments in both the primiparous and multiparous groups, as prepartum diets and management conditions were similar. Postpartum treatments did not affect plasma urea levels of primiparous cows. The interaction between treatment and time of observation was significant ($P < 0.05$), with the control being lower compared to the groups receiving HSLF and HSLF-LSHF concentrates (Figure 3.6). In multiparous cows, HSLF and HSLF-LSHF treatments increased ($P < 0.05$) plasma urea levels, compared to the control (Figure 3.7). However, the interaction between treatment and time of observation on plasma urea levels was not significant in multiparous cows.

Table 3.4 The effect of nutritional treatments differing after calving in energy levels and sources on the plasma urea (mean ± SE) levels recorded for primiparous and multiparous dairy cows during a four week prepartum to 13 week postpartum period.

	Concentrate feeding			P-values		
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[2]**	***W*[2]**	***WT*[2]**
Primiparous cows						
Number of cows	13	6	6			
Prepartum blood urea (mmol/L)	3.37 ± 0.51	3.34 ± 0.71	3.48 ± 0.84	0.74	0.2	NS
Postpartum blood urea (mmol/L)	5.17^{a} ±0.32	5.78^{b} ± 0.38	5.57^{b} ± 0.28	0.62	0.01	0.04
Multiparous cows						
Number of cows	25	19	19			
Prepartum blood urea (mmol/L)	3.54 ± 0.23	3.50 ± 0.30	3.42 ± 0.30	0.08	0.47	NS
Postpartum blood urea (mmol/L)	5.27^{a} ±0.18	6.05^{b} ± 0.22	6.71^{b} ± 0.25	<.001	0.27	NS

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat. [2]*T*: Treatment, *W*: Week, *WT*: Interaction WT.

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

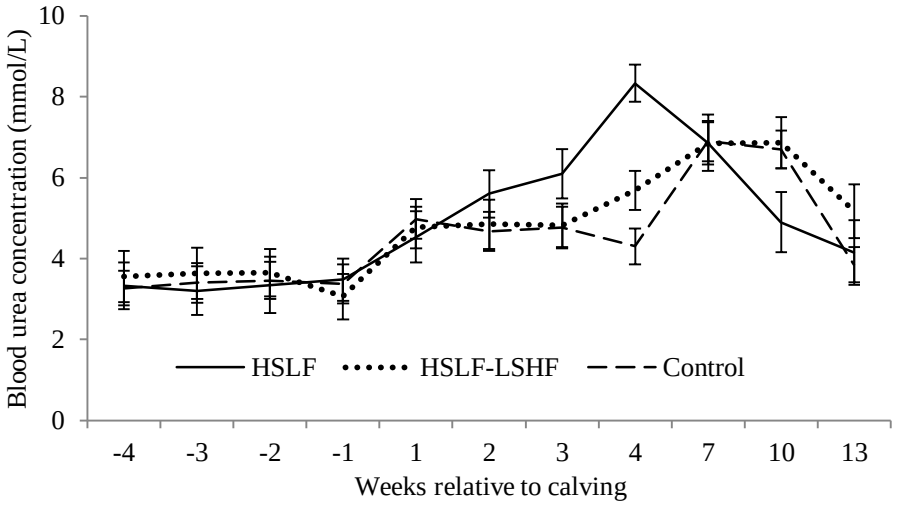

Figure 3.6 The effect of nutritional treatments differing after calving in energy levels and sources on the plasma urea (mean ± SE) levels recorded for primiparous dairy cows during a four week prepartum to 13 week postpartum period.

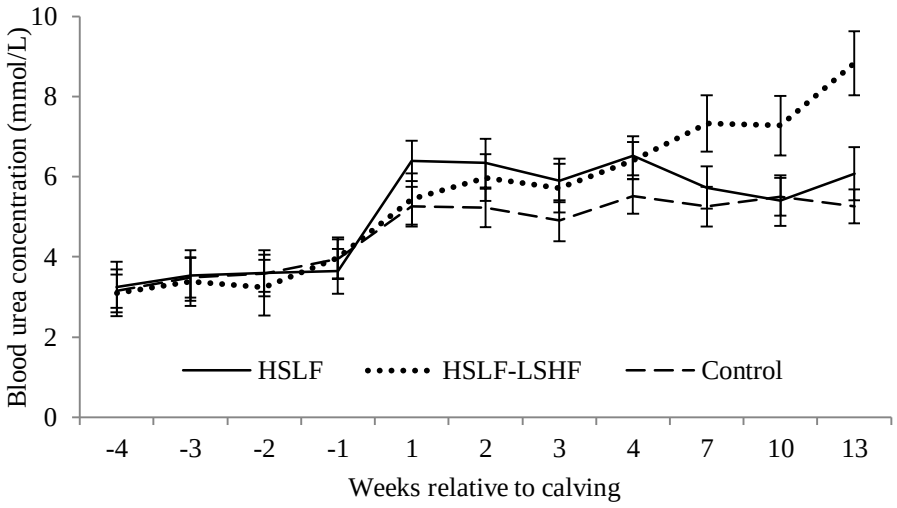

Figure 3.7 The effect of nutritional treatments differing after calving in energy levels and sources on the plasma urea (mean ± SE) levels recorded for multiparous dairy cows during a four week prepartum to 13 week postpartum period.

Different factors contribute to the plasma urea levels measured near to parturition and/or early lactating ruminants. During transition, cows are faced with either energy or protein supplied in the diet being limiting as a result of the requirements for foetal growth or milk production (Wathes *et al*., 2007). As a result, low glucose availability is supplemented by an increased rate of gluconeogenesis that rely on amino acids mobilised from body muscles at the cost of protein synthesis, thus increasing plasma urea production (Bell, 1995; Garnsworthy *et al*., 2008b). Furthermore, plasma urea levels were reported to increase in situations where dietary protein supply exceeds energy availability (Oltner & Wiktorsson, 1983; Moore & Varga, 1996). When the energy supply is restricted, the amount of ammonia production from dietary crude protein exceeds the capability of the rumen microbes to convert it into microbial protein synthesis (MPS) (Wathes *et al*., 2007). This situation increases the circulating plasma ammonia levels, with the excess being converted to urea by the liver (Carlsson & Pehrson, 1994). In previous studies, plasma urea decreased for cows fed a glucogenic diet, compared with cows fed a lipogenic diet (Van Knegsel *et al*., 2007a; Garnsworthy *et al*., 2008a, 2009). However, Van Knegsel *et al*. (2007b) found no differences in blood urea in both primiparous and multiparous cows fed either glucogenic, mixed or lipogenic diets. In contrast, plasma urea levels in this study showed positive relationship with the inclusion levels (high *vs* low) of concentrates, indicating a poor N utilisation in the rumen in HSLF and HSLF-LSHF compared to the control in both parity group (Figures 3.6 and 3.7). Normally, high starch diets improve rumen ammonia capture and decrease plasma urea levels (Reynolds, 2006). The inverse effect in this study can be attributed the structural properties of the pelleted

concentrates impeding the rumen fermentation, when grazing cows were fed high levels of concentrates (i.e. 11.6 kg/day for primiparous and 12.6 kg/day for multiparous cows, respectively) compared to low level of concentrates (i.e. 7 kg/day for both primiparous and multiparous cows). The heat treatment in the pelleting process have probably increased its resistance to rumen fermentation and/or rapidly bypassed the energy nutrients to the small intestine, thus preventing the N capture for MPS in the rumen.

The effects of feeding treatments on LW traits are presented in Table 3.5 for primiparous and multiparous cows, respectively. The effects of year of calving and season of calving on prepartum and postpartum LW as well as on postpartum LW loss were not significant for both primiparous and multiparous groups (therefore, *P*-values of the effect of year of calving was not indicated in Table 3.5). Changes in LW and LW loss over week of observation are illustrated in Figures 3.8 and 3.10 for primiparous cows and Figures 3.9 and 3.11 for multiparous cows, respectively. Prepartum LW was similar for both primiparous (Figure 3.8) and multiparous cows (Figure 3.9), owing to the similar feeding and management conditions during this period. As expected, both pregnant heifers and dry cows gained LW as calving time approached (Figures 3.8 and 3.9; Week of observation $P < 0.05$ not reported in Table 3.5), as would be expected with increased foetal growth during late pregnancy. Approximately 60% in foetal mass, relative to the calf LW at birth, normally occurs in the last two months of the pregnancy (Bauman & Currie, 1980). Through homeorhetic pathway controlling the pregnancy, glucose and amino acids are partitioned not only for the development of the foetus, but also for the growth of the foetal membranes, the gravid uterus, and the mammary gland. In addition, a foetus of a ruminant is more vulnerable than that of many other species to maternal undernutrition stresses, resulting in a severe foetal growth delay (Everitt, 1964). Hence, maternal adaptions in prepartum heifer and cows prioritized nutrients required for their own maternal growth and/or replenishment of protein and energy reserves to meet the foetal requirements. In this study, the lack of differences in prepartum LW of heifers and dry cows can be related to the partitioning of nutrients during pregnancy to support the foetal needs in both parity groups.

Table 3.5 The effect of nutritional treatments differing after calving in energy levels and sources on live weight traits (mean ± SE) recorded for primiparous and multiparous dairy cows during an eight week prepartum to 22 week postpartum period.

	Concentrate feeding			*P*-values		
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[2]**	***S*[2]**	***ST*[2]**
Primiparous cows						
Number of cows	30	20	19	-	-	-
LW from week 8 to 1 prepartum (kg)	522 ± 9	524 ± 11	520 ± 11	0.21	0.81	NS
LW^3 week 1 after calving (kg)	511 ± 14	493 ± 13	491 ± 9	0.43	0.53	NS
LW^3_{nadir} (kg)	429 ± 10	435 ± 9	438 ± 7	0.76	0.77	NS
LW^3 $loss_{nadir}$ (kg)	79^a ± 8	58^b ± 7	54^b ± 5	0.04	0.99	NS
Number of days to reach LW^3_{nadir} (days)	40^a ± 3	26^b ± 2	25^b ± 3	0.003	0.94	NS
Rate of LW loss from calving to LW^3_{nadir} (kg/day)	2.09 ± 0.29	2.43 ± 0.36	2.87 ± 0.37	0.15	0.84	NS
LW^3 at peak MY^3 (kg)	468 ± 10	465 ± 13	463 ± 11	0.94	0.83	NS
Rate of LW gain from LW^3_{nadir} to 154 DIM (kg/day)	0.57 ± 0.04	0.69 ± 0.05	0.60 ± 0.05	0.16	0.35	NS
LW from week 1 to 22 postpartum (kg)	479^a ± 9	488^b ± 11	489^b ± 11	<.0001	0.98	<.0001
LW loss from week 2 to 22 postpartum (%)	6.13^a ± 0.40	-0.92^b ± 27	-0.27^b ± 0.40	<.001	0.87	<.0001
Multiparous cows						
Number of cows	77	38	38	-	-	-
Lactation number	3.82 ± 0.17	3.49 ± 0.22	3.54 ± 0.22	-	-	-
LW from week 8 to 1 prepartum (kg)	660 ± 7	662 ± 7	662 ± 7	0.12	0.41	NS
LW^3 week 1 after calving (kg)	621 ± 8	630 ± 10	620 ± 11	0.11	0.51	NS
LW^3_{nadir} (kg)	541 ± 6	563 ± 9	557 ± 10	0.39	0.59	NS
LW^3 $loss_{nadir}$ (kg)	75 ± 4	68 ± 3	66 ± 3	0.24	0.22	NS
Number of days to reach LW^3_{nadir} (days)	35 ± 2	28 ± 3	29 ± 3	0.07	0.44	NS
Rate of LW loss from calving to LW^3_{nadir} (kg/day)	2.56 ± 0.15	2.85 ± 0.22	2.49 ± 0.21	0.43	0.83	NS
LW^3 at peak MY^3 (kg)	580 ± 7	597 ± 10	595 ± 10	0.23	0.74	NS
Rate of LW gain from LW^3_{nadir} to 154 DIM (kg/day)	0.54 ± 0.03	0.49 ± 0.04	0.48 ± 0.04	0.33	0.37	NS
LW from week 1 to 22 postpartum (kg)	583^a ± 7	603^b ± 7	604^b ± 7	<.0001	0.21	NS
LW loss from week 2 to 22 postpartum (%)	5.85^a ± 0.62	4.18^b ± 0.78	2.73^b ± 0.70	<.001	0.89	<.0001

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat.

[2]*S*: Season, *T*: Treatment, *ST*: Interaction ST. [3]LW: Live weight, MY: Milk yield.

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

In both seasons, higher ($P < 0.05$) postpartum LW in both primiparous and multiparous cows was recorded for HSLF and HSLF-HFLS treatments, compared to the control groups. In primiparous groups, postpartum LW for the control, HSLF and HSLF-HFLS treatments were 482 ± 4; 495 ± 4 and 497 ± 4 kg in winter *vs* 477 ± 3; 480 ± 4 and 481 ± 4 kg in the summer season, respectively. In multiparous groups, postpartum LW for the control, HSLF and HSLF-HFLS treatments were 579 ± 2; 595 ± 3 and 600 ± 2 kg in winter *vs* 588 ± 2; 612 ± 3 and 608 ± 3 kg in the summer season, respectively.

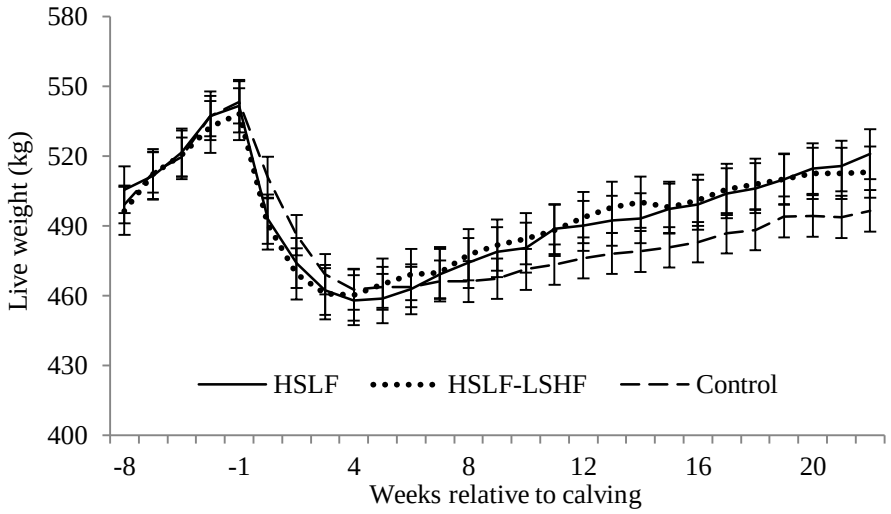

Figure 3.8 The effect of nutritional treatments differing after calving in energy levels and sources on the live weight (mean ± SE) recorded for primiparous dairy cows during an eight week prepartum to 22 week postpartum period.

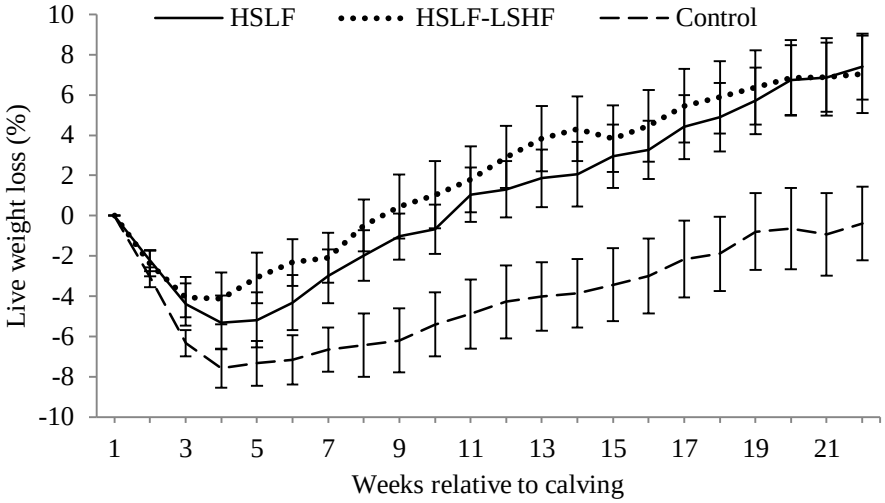

Figure 3.9 The effect of nutritional treatments differing after calving in energy levels and sources on the postpartum live weight loss (mean ± SE) recorded for primiparous dairy cows during a 22 week postpartum period.

The postpartum LW was the lowest ($P < 0.05$) while the LW loss was the highest ($P < 0.05$) in cows receiving the control diet in comparison to HSLF and HSLF-LSHF diets for both primiparous (Figures 3.8 and 3.9) and multiparous cows (Figures 3.10 and 3.11). The interaction between treatment and week of observation on postpartum LW and LW loss was significant ($P < 0.05$) in both primiparous and multiparous groups (*P*-values not indicated in Table 3.5). Also, the interaction between season and treatment on postpartum LW and LW loss was significant ($P < 0.05$) in both primiparous and multiparous cows. The LW $loss_{nadir}$ and the number of days to reach LW_{nadir}

increased ($P < 0.05$) in primiparous cows receiving the control concentrate, compared to HSLF and HSLF-HFLS treatments. This trend was not evident in multiparous cows. A possible explanation of these findings can be attributed to the possible poor hepatic adaptation and increased LW mobilisation in young and still growing primiparous cows to postpartum energy stress, compared to mature multiparous cows (parity > 3).

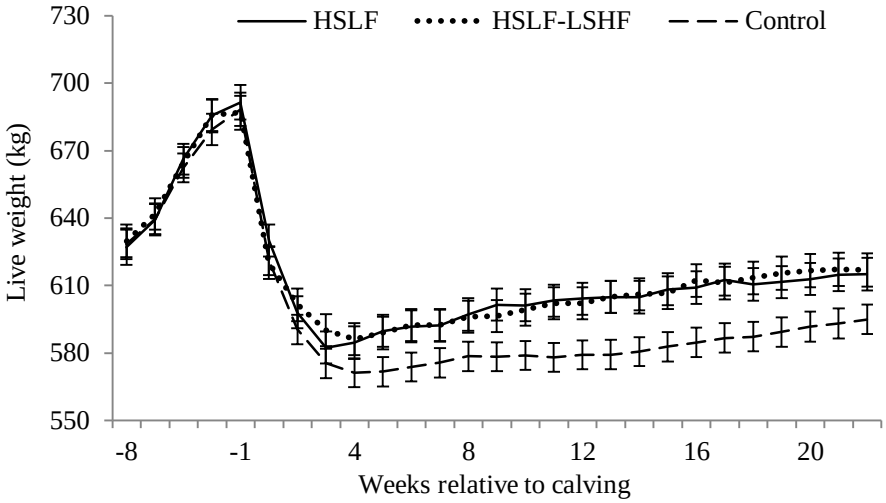

Figure 3.10 The effect of nutritional treatments differing after calving in energy levels and sources on the live weight (mean ± SE) recorded for multiparous dairy cows during an eight week prepartum to 22 week postpartum period.

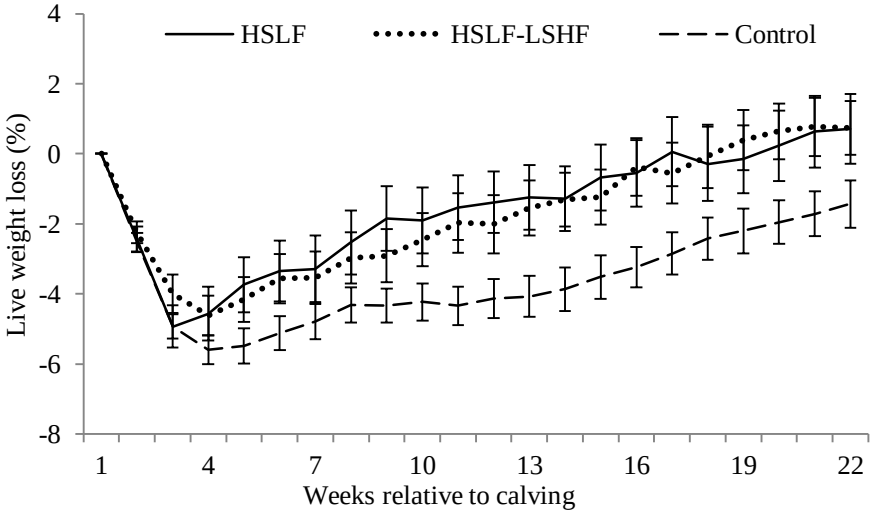

Figure 3.11 The effect of nutritional treatments differing after calving in energy levels and sources on the postpartum live weight loss (mean ± SE) recorded for multiparous dairy cows during a 22 week postpartum period.

Following parturition, Tamminga *et al.* (1997) found that the energy partitioning into milk from the mobilisation of body fat and protein reserves resulted in a LW loss in cows fed on DM basis 11.1

kg/day of roughage and 10.8 kg/day of concentrates. Within the eight weeks of the trial period, cows mobilised an average of 0.7 kg LW, being 0.56 kg fat and 0.04 kg protein per day. The LW loss due to this mobilisation was highest in the first week after calving and decreased as the lactation period progressed (Tamminga *et al*., 1997). Previous studies also reported LW losses during the postpartum period (Senatore *et al*.**,** 1996; Garnsworthy, 2002; Buckley *et al*., 2003; Lucy, 2007; Sakaguchi, 2009). However, other studies found no effects on LW (Van Knegsel *et al*., 2007a; Garnsworthy *et al*., 2009), probably because of the isocaloric diets used in the studies. In the current study, postpartum LW of cows in both parity groups decreased until the nadir point in early lactation and increased afterwards. During early lactation, the shift of the somatotropic axis towards improved milk synthesis was reported to be associated with the increased prioritization of nutrients to satisfy the considerable demands of the mammary gland at the expense of body reserves and LW (Leroy *et al*., 2008). Jorritsma *et al*. (2003) reported that a postpartum cow undergoing continuous weight loss over time is definitely in a state of NEB, while a cow that gains weight over time is preventing a mobilisation of body reserves to subsequently overcome a NEB. After the LW_{nadir}, cows that are maintaining or gaining LW demonstrate a positive indicator towards process of stabilising or improving the EB status in these cows (Van Straten *et al*., 2008). Consistent with these arguments, postpartum LW of cows in this study decreased in the control group in comparison to HSLF and HSLF-LSHF treatments in both parity groups, indicating differences in nutritional status between treatments as the lactation progressed. This finding showed that abnormal deviations in LW over time could be utilised as a proxy to identify the physiological well-being and the nutritional status of each cow in the herd in response to managerial purposes (e.g. nutritional treatment according to performance or to prevent undesired LW loss or gain). Bauman and Currie (1980) reported that improvements in nutritional status of lactating cows resulted in changes in levels of plasma metabolites (i.e. glucose, lipids, amino acids, etc.) and an increase in the ratio of insulin to glucagon in the pancreas. These changes resulted in a greater hepatic uptake of metabolites, supporting the milk synthesis in the udder with the excess being diverted to peripheral circulation of cows. Such diversion of metabolites, occurring when the EB is improved in cows, increased the synthesis of glycogen in the liver, lipids in the adipose tissue and protein in the muscle, all together replenishing the postpartum body reserves and LW. In this study, the difference in postpartum LW in both parity groups can be attributed to changes in the somatotropic responses to the total nutrient and energy intakes from nutritional treatments in coordinating the mobilisation of body mass as a physiological mechanism to adapt to the energy deficit, while sustaining milk production.

3.4. Conclusion

In this study, all cows mobilised their body reserves before and after calving. Compared to the control, HSLF and HSLF-LSHF treatments significantly decreased plasma NEFA and BHB levels in primiparous cows and increased plasma urea levels in both primiparous and multiparous cows. In addition, HSLF and HSLF-LSHF treatments significantly decreased postpartum LW loss of primiparous and multiparous cows in comparison to the control. These biological responses showed an enhanced nutritional status of dairy cows in HSLF and HSLF-LSHF treatments, probably favouring somatotropic axis and metabolic responses that benefit the restoration of body reserves and LW, in comparison to the control. Further research is needed to investigate the point at which long-term changes in postpartum LW (i.e. LW $loss_{nadir}$ and number of days to reach the LW_{nadir}) act as stressors to disrupt milk responses, suppress the gonadotropic axis, and reduce fertility outcomes of dairy cows.

3.5. References

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Chapter 4

Effect of energy sources on the milk production and milk composition of Holstein cows

Abstract

The objective of the study was to evaluate the effect of supplementing different energy sources and levels on the milk production and milk composition of Holstein cows in a pasture-based feeding system. A total of 69 primiparous and 153 multiparous Holstein cows from the Elsenburg Research Farm were allocated to three postpartum nutritional treatments according to parity, calving date, live weight (LW) at calving and milk yields in the previous lactation. The nutritional treatments consisted of *ad libitum* access to cultivated irrigated kikuyu-ryegrass pastures, supplemented with different levels and types of concentrates containing starch and/or fat. The control cows received a low level of a control supplement, while treatments consisted of high levels of supplements containing high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) energy contents. Maize, being a glucogenic feed ingredient, was the main energy source in HSLF supplement. The supplements in treatment HSLF-LSHF were combinations of a glucogenic concentrate offered for the first 60 days in milk (DIM) as per treatment HSLF and followed from 61 DIM by a lipogenic concentrate containing wheat bran and calcium (Ca)-salts of long-chain fatty acids as the energy sources. Milk yield and 4% fat corrected milk (FCM) yield in both primiparous and multiparous cows receiving HSLF and HSLF-HFLS concentrates were higher ($P < 0.05$), compared to cows receiving the control concentrate supplement. In both parity groups, HSLF and HSLF-HFLS treatments increased ($P < 0.05$) the milk yield at peak of lactation in comparison to the control, but the number of days at peak of lactation was similar between nutritional treatments. In the primiparous group, the HSLF diet improved milk protein content ($P < 0.05$) whereas HSLF and HSLF-HFLS diets resulted in increased milk lactose content ($P < 0.05$). In multiparous group, only the milk lactose content was improved ($P < 0.05$) in the HSLF treatment. Milk yield, milk fat percentage and milk urea nitrogen (MUN) concentration were higher ($P < 0.05$) for cows calving in winter in comparison to cows calving in the summer season. Results indicated that manipulating energy-based concentrates containing starch and/or fat probably improved the profile of the total energy and nutrients absorbed from the gastro-intestinal tract (GIT), the partitioning of the digesta in the liver, and the somatotropic axis of dairy cows. Such enhancements in dairy cows consequently increased overall milk production and altered the milk composition.

4.1. Introduction

Milk productivity of dairy cows has increased considerably during the last few decades (Lucy, 2001). Given that the quantity and the quality of dry matter (DM) intake significantly affect milk production responses (Bargo *et al*., 2003; Hills *et al*., 2015), it is the manner, in which dairy cows shift from the dry period (DP) to the onset of the lactation, that is a key factor in their subsequent milk yield and milk composition (McArt *et al.*, 2013). Usually in early lactation, the increased milk production exceeds the DM intake in dairy cows (Gilmore *et al*., 2011). This condition often leads to the establishment of a negative energy balance (NEB), which results in the mobilization of body reserves (Hammon *et al*., 2009) as a physiological mechanism to meet the energy demand for maintenance and milk production during early lactation (Rukkwamsuk *et al*., 1999; Jorritsma *et al*., 2003).

Common postpartum strategies that aim for an increased energy intake while decreasing the extent of a NEB status consist of adding energy-dense ingredients like fat and/or non-fibre carbohydrates into diets (Garnsworthy *et al*., 2008a, b; Pedernera *et al*., 2008). Non-structural carbohydrates, primarily starch, are usually increased in the diet through the inclusion of grains (Dyck *et al*., 2011). Dietary fats consist of ruminally inert sources (i.e. hydrogenated fish fat, Ca-salts of long-chain fatty acids, high melting point fatty acids) or non-ruminally inert sources (i.e. full fat rapeseed and soybean oil) (Bargo *et al*., 2003). Importantly, feeding a starch-based diet enhances the supply of glucogenic precursors to increase circulating insulin levels (Van Knegsel *et al.*, 2007b) and enhances the protein/energy balance to potentially improve the microbial protein synthesis (MPS) in the rumen (Rearte & Pieroni, 2001). However, feeding excessive amounts of cereal grains to dairy cows can increase the risk of ruminal acidosis, decrease the acetate/propionate ratio, reduce ruminal fibre digestibility and decrease milk fat concentration (Bargo *et al*., 2003; Bannink *et al*., 2006). Adding fat into dairy diets increases the energy density of the diet, improves the metabolic energetic efficiency and alters milk fat level and composition (Palmquist, 1994; Weis & Pinos-Rodriguez, 2009). Of interest among fats are the Ca-salts of the long-chain fatty acids that are insoluble at normal rumen pH and have inert properties on rumen microbial digestion (Chalupa *et al*., 1986; Voigt *et al*., 2003). In the abomasum, these fats are broken down by hydrochloric acid into free fatty acids and Ca-ions. The rumen bypass of these fats consequently increases their absorption from the small intestine, thus enhancing the energy intake and the delivery of polyunsaturated fatty acids to the mammary gland (Palmquist, 1994; Purushothaman *et al*., 2008; Weis & Pinos-Rodriguez, 2009).

Optimal provision of such dietary starch and/or fat into postpartum diets of dairy cows have been reported to have implications on rumen function, absorbed nutrients through the GIT, energy partitioning, milk yield and milk composition, endocrine system and reproductive outcomes (Garnsworthy *et al*., 2008a, b; Roche *et al*., 2011, 2013, Esposito *et al*., 2014). The assumption of this study was that manipulating supplements differing in energy levels and sources to cows grazing on pastures can improve nutrient and energy intake to subsequently improve milk production. Therefore, the objective of this study was to investigate the effect of supplementation differing in energy levels (low *vs* high) and sources (starch *vs* fat) on milk production and milk composition of Holstein cows in a pasture-based feeding system.

4.2. Materials and Methods

4.2.1. Experimental location

The trial was conducted at the Elsenburg Research Farm, as described in Chapter 3 (3.2.1).

4.2.2. Experimental husbandry, animals and diets

Fresh drinking water was freely available at all times in the trial. The same animals (69 primiparous and 153 multiparous Holstein cows) and experimental diets as described in Chapter 3 (3.2.2) were used. Briefly, all fresh cows were allocated following parturition to three isonitrogenous postpartum nutritional treatments according to parity, calving date, LW at calving and milk yields of the previous lactation. The nutritional treatments consisted of pastures supplemented with pelleted concentrates containing energy sources such as starch and/or fat. The Kikuyu-ryegrass pastures were irrigated using a permanent irrigation system. Cows followed a rotational programme to ensure an *ad libitum* DMI. Due to the experimental design, it was not possible to determine group pasture intake of cows in the different treatments, since cows grazed pastures as one herd for the duration of the experiment. The control contained low levels of a control supplement, while the HSLF and HSLF-LSHF treatments consisted of high levels of supplements. The control was the standard pasture feeding system, with an allowance of a control concentrate of 7 kg/day from calving until 154 DIM for both the primiparous and multiparous cows. The control diet contained low level of energy content (2.47 Mcal ME/kg DM) and provided 457, 104 and 40 g/kg of NDF, starch and fat on DM basis, respectively. The concentrate allowances in treatments HSLF and HSLF-LSHF were 11.6 kg/day for primiparous and 12.6 kg/day for multiparous cows, respectively. The supplement in treatment HSLF was a glucogenic concentrate containing maize as the energy source and was offered from calving to 154 DIM. The HSLF diet contained high level of energy

content (2.71 Mcal ME/kg DM) and offered 341, 242 and 35 g/kg of NDF, starch and fat on DM basis, respectively. The HSLF treatment was fed to increase plasma insulin and glucose levels in order to reduce the extent and duration of a NEB.The supplements in treatment HSLF-LSHF were combinations of a glucogenic concentrate offered for the first 60 DIM as per treatment HSLF and followed from 61 to 154 DIM by a lipogenic (LSHF) concentrate, using wheat bran and Ca-salts of long-chain fatty acids (Megalac rumen bypass fat, Volac International Ltd., UK) as the energy sources. In the HSLF-LSHF combination treatment, the high starch-based diet initially aimed to achieve the same objective of the HSLF treatment during the first 60 DIM. The LSHF diet contained high level of energy content (2.70 Mcal ME/kg DM) and provided 388, 137 and 58 g/kg of NDF, starch and fat on DM basis, respectively. Secondly, the LSHF diet was fed from 61 DIM to improve the EB status using a rumen bypass fat at an inclusion level of 58 g/kg on DM basis, while decreasing plasma insulin level and improving plasma cholesterol level. Half of the concentrate allowance was fed after each milking and cows were milked twice a day at 05:30 and 15:00.

4.2.3. Experimental sampling and data collection

Calving seasons were summer from 15th October to 14th April, with December to February being the hottest months and winter from 15th April to 14th October, with June to August being the coldest months. Daily milk yields of cows were automatically recorded (Afikim system, AfiMilk) after each milking and pooled to obtain weekly means. Milk samples were collected at the evening and following morning's milking sessions every 35 days, preserved with 2-bromo-2-nitropropane-1, 3 diol and analysed for milk fat, milk protein, milk lactose and MUN levels by infrared spectrophotometry (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). Milk composition data were pooled to obtain test means at a 35 days interval, respectively. The test periods were from 1 to 35, 36 to 70, 71 to 105, 106 to 140 and 141 to 154 DIM, respectively. The 4% FCM yield was calculated based on the equation of Gaines (1928): 4% FCM, kg/d = (0.4 x milk yield, kg/d) + (15 x fat, kg/d).

4.2.4. Statistical analysis

Data were analysed using the PROC MIXED of SAS enterprise guide (SAS, 2012), grouped according to parity i.e. primiparous or multiparous cows. The statistical model included year (*Y*) effect, calving season (*S*) effect, treatment (*T*) effect, week (*W*) effect of observations and the interaction effects between treatment and week of observation (*WT*) as well as treatment and calving season (*ST*) as fixed effects. The animal effect within treatments was specified as a random effect. The effects of year and season of calving were included in the statistical model because of

the duration of the experiment. The measured variables obtained every day within a particular week during the trial were considered as repeated observations of that particular week block [chapter 3 (3.2.4) for justification]. The statistical model was as follows:

$Model = \mu + Y_i + S_j + T_k + W_l + (ST)_{jk} + (WT)_{kl} + \delta_{(lk)m} + \varepsilon_{ijklm}$, where

μ = overall mean;

Y_i = the fixed effect of the i^{th} year of calving (i.e. 2012, 2013 and 2014);

S_j = the fixed effect of the j^{th} season of calving (i.e. summer and winter);

T_k = the fixed effect of the k^{th} treatment (i.e. Control, HSLF and HSLF-LSHF);

W_l = the fixed effect of the l^{th} week of observation (i.e.1 to 22)

$(ST)_{jk}$ = the interaction between levels of the j^{th} season of calving and k^{th} treatment;

$(WT)_{kl}$ = the interaction between levels of the l^{th} week of sampling and k^{th} treatment;

$\delta_{(kl)m}$ = the variable effect of the m^{th} block effect in the k^{th} treatment (Repeated statement);

ε_{ijklm} = the random experimental error.

All effects were used to analyse the milk and 4% FCM yields whereas the milk composition traits (i.e. milk fat, milk protein, milk lactose and MUN) were analysed without the repeated statement ($\delta_{(kl)m}$) in the statistical model. The milk yield at peak of lactation and the number of days at peak of lactation were analysed without the repeated statement ($\delta_{(kl)m}$), but with the year, calving season, treatment, as well as the interaction between calving season and treatment as fixed effects in the statistical model. Statistical assumptions were described as fixed effects and their interactions were equal to zero with $\delta_{(kl)m} \sim N(0, \sigma_e^2)$ varying independently of ε_{ijklm}. Differences of means and standard error (SE) of means between treatments were obtained using the pair wise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

4.3. Results and Discussion

The effects of the inclusion level and type of energy supplement on milk production and milk composition of grazing primiparous and multiparous cows on kikuyu-ryegrass pastures are presented in Table 4.1. The effect of nutritional treatments differing in energy levels and sources on milk yields over time are presented in Figures 4.1 and 4.2 for primiparous and Figures 4.3 and 4.4 for multiparous cows, respectively.

Table 4.1 The effect of nutritional treatments differing after calving in energy levels and sources on the milk production and milk composition (mean ± SE) recorded for primiparous and multiparous dairy cows during a 22 week postpartum period.

	Concentrate feeding			*P*-values				
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[2]**	***W*[2]**	***S*[2]**	***ST*[2]**	***TW*[2]**
Primiparous cows								
Number of cows	30	20	19					
Milk yield (kg/day)	19.6[b] ± 0.46	21.9[a] ± 0.63	22.0[a] ± 0.60	<.0001	<.0001	0.0008	<.0001	0.03
4% fat corrected milk yield (kg/day)	20.5[b] ± 0.25	23.0[a] ± 0.34	22.6[a] ± 0.32	0.005	<.001	0.001	<.001	0.04
Milk yield at peak of lactation (kg/day)	26.6[b] ± 0.59	29.5[a] ± 0.67	29.7[a] ± 0.68	0.04	-	0.21	NS	-
Number of days to reach peak milk yield (days)	31 ± 3	27 ± 3	31 ± 3	0.53	-	0.98	NS	-
Fat (%)	3.75 ± 0.07	3.91 ± 0.07	3.74 ± 0.06	0.13	<.001	0.0002	0.03	NS
Protein (%)	3.10[a] ± 0.05	3.26[b] ± 0.05	3.13[a] ± 0.05	0.04	0.0005	0.42	NS	NS
Lactose (%)	4.84[a] ± 0.03	4.95[b] ± 0.03	4.91[b] ± 0.03	0.04	0.008	0.34	NS	NS
Fat: Protein ratio	1.22 ± 0.02	1.21 ± 0.02	1.20 ± 0.02	0.78	0.12	0.001	NS	NS
Milk Urea Nitrogen (mg/kg)	15.0 ± 0.66	15.0 ± 0.66	15.1 ± 0.62	0.98	<.0001	0.0031	NS	NS
Multiparous cows								
Number of cows	77	38	38					
Lactation number	3.82 ± 0.17	3.49 ± 0.22	3.54 ± 0.22	-	-	-	-	-
Milk yield (kg/day)	26.3[b] ± 0.40	29.4[a] ± 0.63	29.2[a] ± 0.42	<.0001	<.0001	<.0001	0.0012	<.001
4% Fat corrected milk yield (kg/day)	27.6[b] ± 0.23	30.5[a] ± 0.28	30.6[a] ± 0.29	<.001	<.001	0.003	0.0017	<.001
Milk yield at peak of lactation (kg/day)	38.5[b] ± 0.67	41.3[a] ± 0.73	40.8[a] ± 0.80	0.014	-	0.02	NS	-
Number of days to reach peak milk yield (days)	32 ± 2	33 ± 2	32 ± 2	0.77	-	0.12	NS	-
Fat (%)	3.78 ± 0.03	3.78 ± 0.05	3.77 ± 0.05	0.98	<.0001	0.005	NS	NS
Protein (%)	3.07 ± 0.03	3.11 ± 0.04	3.13 ± 0.04	0.27	<.0001	0.64	NS	NS
Lactose (%)	4.71[a] ± 0.02	4.82[b] ± 0.03	4.72[a] ± 0.02	0.0004	0.0052	0.017	0.017	NS
Fat: Protein ratio	1.24 ± 0.01	1.22 ± 0.01	1.21 ± 0.01	0.24	0.02	0.0005	0.007	NS
Milk Urea Nitrogen (mg/kg)	13.7 ± 0.35	14.5 ± 0.53	14.9 ± 0.49	0.07	<.0001	<.0001	NS	NS

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat.[2]*S*: Season of calving, *W*: week of observation, *T*: Treatment, *ST*: Interaction ST, *TW*: Interaction TW.

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

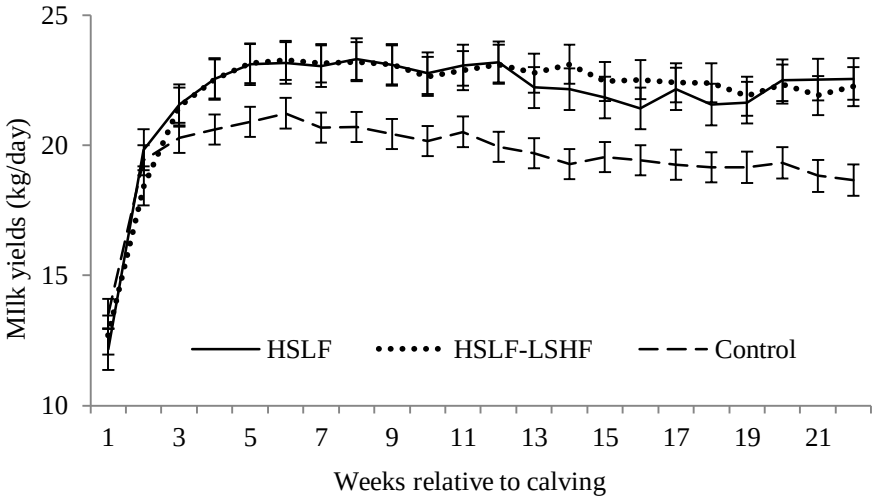

Figure 4.1 The effect of nutritional treatments differing after calving in energy levels and sources on the milk yield (mean ± SE) recorded for primiparous dairy cows during a 22 week postpartum period.

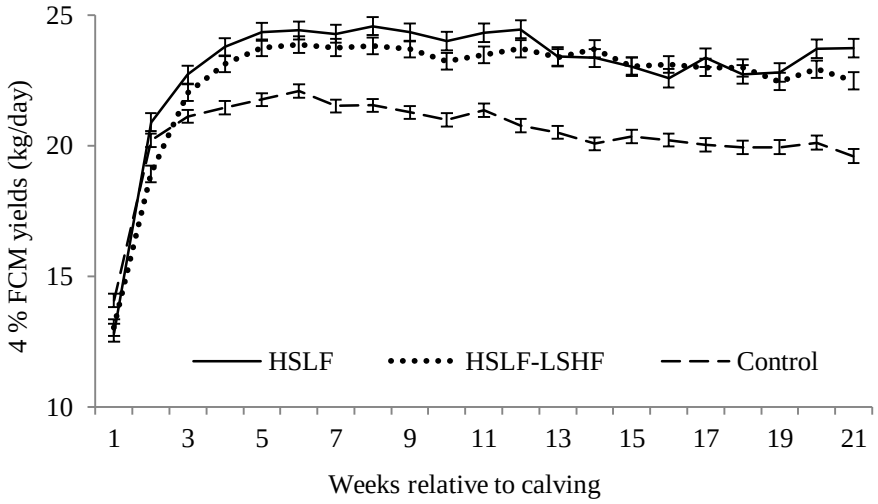

Figure 4.2 The effect of nutritional treatments differing after calving in energy levels and sources on the 4% fat corrected milk (FCM) yield (mean ± SE) recorded for primiparous dairy cows during a 22 week postpartum period.

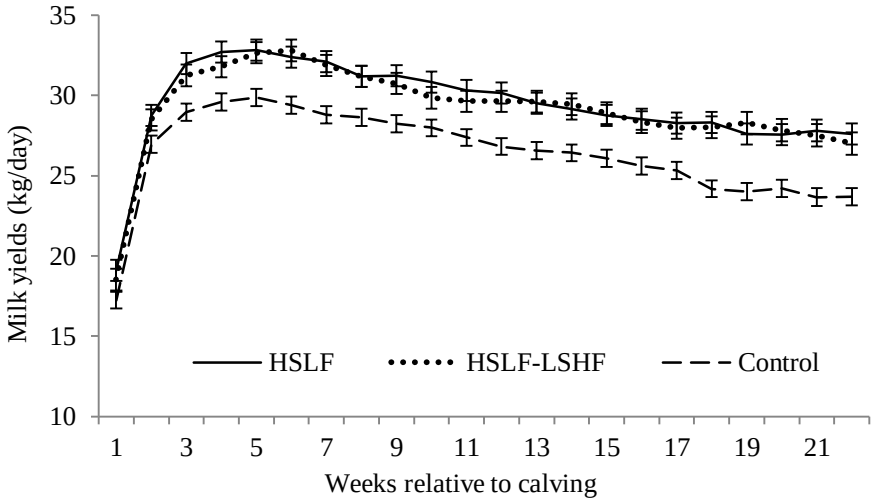

Figure 4.3 The effect of nutritional treatments differing after calving in energy levels and sources on the milk yield (mean ± SE) recorded for multiparous dairy cows during a 22 week postpartum period.

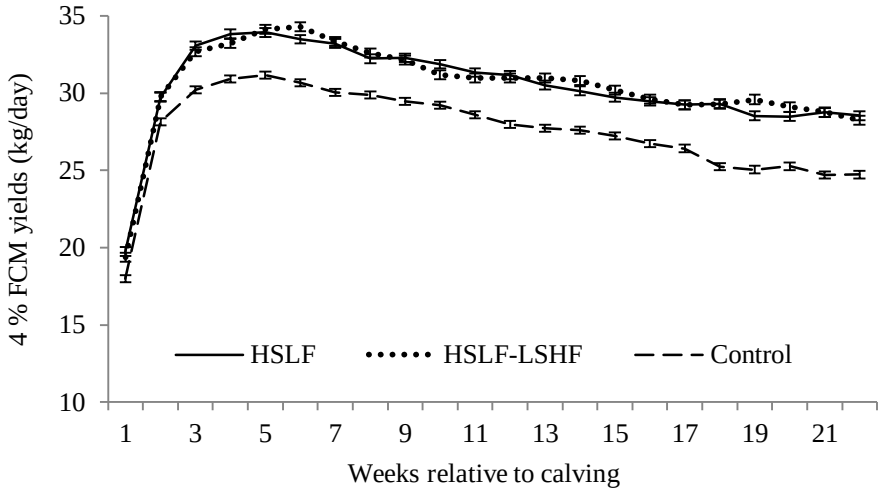

Figure 4.4 The effect of nutritional treatments differing after calving in energy levels and sources on the 4% fat corrected milk (FCM) yield (mean ± SE) recorded for multiparous dairy cows during a 22 week postpartum period.

The year effect on milk production and milk composition was not significant for both primiparous and multiparous cows (therefore *P*- values are not included in Table 4.1). The lack of year effect suggests that nutritional treatments did not differ yearly during the trial, thus nullifying its effect in the model. The milk yields and 4% FCM yields in both primiparous and multiparous cows were higher ($P < 0.05$) in HSLF and HSLF-HFLS treatments, compared to the control group. Also, milk yields increased over time (week of observation, $P < 0.05$), while interaction effects between nutritional treatments and time of observations were detected ($P < 0.05$) for both primiparous and multiparous cows. The milk yield of both primiparous and multiparous cows calving in winter was

higher ($P < 0.05$, Table 4.1) compared to cows calving in the summer season. In both seasons, higher ($P < 0.05$) milk yields in both primiparous and multiparous cows were recorded for HSLF and HSLF-HFLS treatments, compared to the control groups. In the primiparous groups, milk yields for the control, HSLF and HSLF-HFLS treatments were 20.2 ± 0.46; 22.7 ± 0.64 and 23.0 ± 0.64 kg/day, respectively, in the winter *vs* 19.0 ± 0.41; 21.3 ± 0.63 and 21.0 ± 0.54 kg/day in the summer. In the multiparous groups, milk yields for the control, HSLF and HSLF-HFLS treatments were 27.6 ± 0.16; 30.1 ± 0.25 and 30.2 ± 0.21 kg/day, respectively, in the winter *vs* 25.1 ± 0.17; 28.7 ± 0.26 and 28.3 ± 0.17 kg/day in the summer. Mostert *et al*. (2001) also reported higher milk yields in dairy cows calving during the cooler months in South Africa. Similarly, other studies found that dairy cows under Mediterranean conditions that calved in summer produced less milk per lactation than those calving in the winter season (Barash *et al*., 1996; Kadzere *et al*., 2002). Additionally, HSLF and HSLF-HFLS treatments increased ($P < 0.05$) the milk yield at peak of lactation in comparison to the control in both primiparous and multiparous cows, but the number of days to reach peak of lactation was similar between nutritional treatments (Table 4.1). However, the season and the interaction between seasons and treatments did not affect the milk yield at peak of lactation and the number of days to reach peak of lactation. The level and type of energy supplementation had a significant effect on milk composition ($P < 0.05$). In primiparous cows, the HSLF diet resulted in increased milk protein content ($P < 0.05$; Table 4.1) whereas HSLF and HSLF-HFLS diets resulted in an increase in milk lactose content ($P < 0.05$; Table 4.1). In multiparous cows, the only significant effect of the diet was observed with supplementation with HSLF concentrates, where milk lactose content was improved (Table 4.1). Calving season and its interaction with the treatments ($P < 0.05$) had an effect on milk composition. A higher milk fat and MUN contents were detected in milk samples in the winter period, when compared to milk samples produced in the summer season ($P < 0.05$; Table 4.1). This was in agreement with studies reporting changes in milk composition of dairy cows as result of changes in seasons of calving (Mostert *et al*., 2001; Kadzere *et al*., 2002). Supporting the current results of this study on the productive performances of dairy cows, other studies found that feeding either extra glucogenic or lipogenic nutrients significantly improved milk yields (Van Knegsel *et al*., 2005; Reis *et al*., 2012; Roche *et al*., 2013) and affected milk composition (Erickson *et al*., 1992; Harrison *et al*., 1995; Chouinard *et al*., 1997; Voigt *et al*., 2003; Van Knegsel *et al*., 2007c).

Maintaining dairy cows on pastures decreases cost of feeding and production as grazed forage is the cheapest source of nutrients (Clark & Kanneganti, 1998; Peyraud & Delaby, 2001). However, pastures as the only diet do not meet all nutrient requirements of dairy cows, especially during early

lactation (Bargo *et al.*, 2003) as a result of the low DM content and high content of highly degradable CP in relation to non-structural carbohydrates (Delahoy *et al*., 2003). In several parts of the world, grain or pelleted concentrates are fed to dairy cows in the milking parlour to compliment pastures. Reports on this feeding strategy showed that the levels and types of concentrates offered in the milking parlour during or after milking times improved milk production responses (Doyle *et al*., 2001; Bargo *et al.*, 2003, Auldist *et al.*, 2013). Furthermore, Higgs *et al*. (2013) reported that supplementing either starch and/or fat-based concentrates in a pasture based system increases milk production as a result of increased metabolizable energy (ME). Supporting this argument, other investigations found that either feeding extra glucogenic or lipogenic nutrients similarly improved milk yields (Van Knegsel *et al*., 2005; Reis *et al*., 2012; Roche *et al*., 2013). To the contrary, some other studies showed no effects on milk yield when feeding either starch- or fat-based diets or their combinations to dairy cows (Garnsworthy *et al*., 2009; Gilmore *et al*., 2011; Little *et al*., 2016). These researchers suggested that the lack of milk response was attributed to the use of isocaloric diets in their studies. In contrast to these studies, the present study was designed to provide two isocaloric concentrates at high levels (i.e. 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively) with a contrast in glucogenic and lipogenic nutrient contents in HSLF and HSLF-LSHF treatments, compared to a lower concentrate level (i.e. 7 kg/day for both primiparous and multiparous cows) in the control group. As a result, one possible explanation in the current study for the improved milk production with HSLF and HSLF-LSHF treatments can be attributed to the concentrate levels that were fed in different nutritional treatments. A greater quantity of concentrates fed potentially increased the total nutrient and ME intake as well as their hepatic partitioning in cows (Bargo *et al*., 2003; Hills *et al.*, 2015), positively affecting the somatotropic axis (i.e. GH, the GH receptor and IGF-I) for high milk synthesis in the mammary gland (Chagas *et al*., 2007). Such enhancement in nutrient and ME intake was reported to affect the lactation persistence thereafter positively (Hermansen, 1990; Reis *et al*., 2012). Furthermore, glucogenic nutrients, like in the HSLF treatment, are fermented in the rumen to produce propionate or bypassed to the small intestine, which efficiently improves the synthesis of glucose in the liver (Steinhour & Bauman, 1988), compared to the control diet. Greater glucogenesis was found to enhance lactose synthesis in the mammary gland and increased water movement into the mammary secretory cells, which both subsequently led to a greater milk volume (Voigt *et al*., 2003; Hills *et al*., 2015). In the HSLF-LSHF treatment, the benefits of LSHF diet on milk yield can be attributed to its increase energy density (Coppock & Wilks, 1991), without necessarily any increase of glucose synthesis or amino acid supply (Weiss & Pinos-Rodriguez, 2009), compared to the control diet. The

improvement in energy density in this diet is related to the fat content because lipids have three times more net energy of lactation than protein and starch (Schroeder *et al*., 2004). Supporting the current increase in milk yield in this study, previous research also reported improved milk production as a result of energy supplementation (Erickson *et al*., 1992; Chouinard *et al*., 1997; Moallem *et al*., 2000; Bargo *et al*., 2003, Hills *et al*., 2015).

Other possible explanations for enhanced milk response to concentrate supplementation are probably related to the stage of lactation and the genetic merit of the dairy cows. Typically, cows in the declining stage of lactation tend to allocate more energy to restoring body tissues that were mobilized to overcome energy deficits during periods of NEB and peak milk yields (NRC, 2001). Macoon *et al*. (2011) reported that milk production was not affected following a supplementation of energy dietary during mid- to late lactation, because the energy intake was being prioritized to restore body tissues. In contrast, results of this study showed improvement in milk production when concentrates were fed from calving until 154 DIM at high levels (i.e. 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively) in HSLF and HSLF-LSHF treatments compared to the low level (i.e. 7 kg/day for both primiparous and multiparous cows) in the control in a pasture based system. Consistent with this response, milk production during early lactation improved with increased levels of supplements fed to cows grazing on pastures (Roche *et al*., 2013). Furthermore, a higher milk yield response was likely to occur when high genetic merit cows (> 25 kg/day of milk during early lactation) were fed high levels of energy supplementation from the early stage of lactation (Kellaway & Porta, 1993; Garnsworthy, 2002; Bargo *et al*., 2003). During this period, high genetic merit cows lose more LW and partition more energy and nutrients towards the udder (Hills *et al*., 2015) to sustain high milk persistence than low genetic merit cows (Snijders *et al*., 2001). Due to the postpartum partitioning of nutrients and ME towards milk production in early lactation, the milk response to supplementation is higher than in the late lactation, a period when more nutrients are partitioned towards body weight gain (Kellaway & Porta, 1993).

The effects of the energy type and level of supplements on milk lactose contents are presented in Figure 4.5 for primiparous and Figure 4.6 for multiparous cows, respectively. The calving season effect and the interaction effect between calving season and treatment affected *($P < 0.05$)* the levels of milk lactose in the multiparous cows, whereas no effects were detected for the primiparous cows (Table 4.1). Levels of milk lactose contents for the control, HSLF and HSLF-HFLS treatments were respectively 4.84 ± 0.04; 4.78 ± 0.03 and 4.71 ± 0.02% in the winter *vs* 4.80 ± 0.03; 4.66 ± 0.03 and 4.72 ± 0.02% in the summer in multiparous groups.

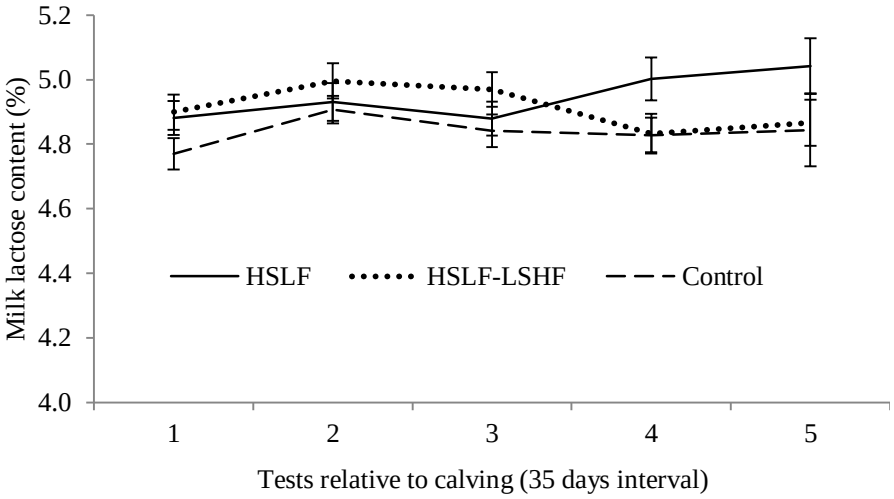

Figure 4.5 The effect of nutritional treatments differing after calving in energy levels and sources on the milk lactose content (mean ± SE) recorded for primiparous dairy cows at a 35 days interval during a 154 day postpartum period.

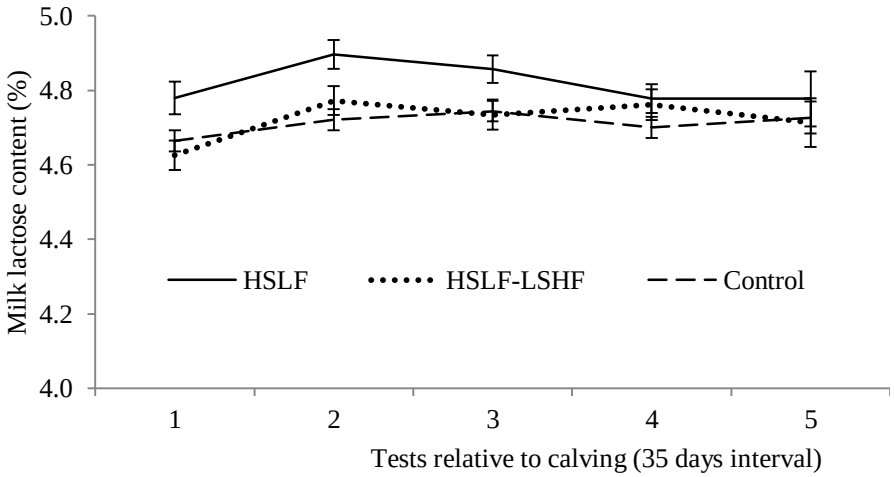

Figure 4.6 The effect of nutritional treatments differing after calving in energy levels and sources on the milk lactose content (mean ± SE) recorded for multiparous dairy cows at a 35 days interval during a 154 day postpartum period.

Additionally, HSLF and HSLF-LSHF treatments increased (*P = 0.04*) milk lactose content in the primiparous cows, compared to the control (i.e. 4.95, 4.91 *vs* 4.84%; Table 4.1 and Figure 4.5). In multiparous cows, high milk lactose was also recorded (*P <.001*) for HSLF treatment, compared the HSLF-LSHF and the control (i.e. 4.82, 4.72 *vs* 4.71%; Table 4.1 and Figure 4.6). In addition, no effects for the interaction between treatment and time of observation were recorded regarding milk lactose content in both parity groups. Consistent with this response, milk lactose content of dairy cows increased with dietary starch, but decreased with dietary fat in the diet (Van Knegsel *et al.*, 2007c). However, other studies reported no effect on milk lactose content when either starch or fat

were added to the diets (Van Knegsel *et al*., 2007a; Garnsworthy *et al*., 2008a, b, 2009, Dyck *et al*., 2011). The observed increased milk lactose in this study could possibly be related to subsequent metabolism towards glucogenesis with starch-based diets (Williams & Stanko, 2000; Garnsworthy *et al*., 2008a), as starch is either fermented in the rumen to stimulate the production of propionate or bypassed the rumen and is absorbed in the small intestine, all together stimulating the production of glucose in the liver (McDonald *et al.*, 2002). Dyck *et al*. (2011) noted that pathways enhancing the hepatic glucose synthesis ultimately improve lactose synthesis in the udder, which subsequently enhances milk lactose content.

The effects of the energy type and level of supplements on milk fat contents are illustrated in Figure 4.7 for primiparous and Figure 4.8 for multiparous cows, respectively. Calving season and the interaction between calving season and treatment affected ($P < 0.05$) milk fat content, with low levels in summer, compared to the winter season in both primiparous and multiparous groups. In primiparous groups, levels of milk fat contents for the control, HSLF and HSLF-HFLS treatments were respectively 3.79 ± 0.08; 4.04 ± 0.10 and 3.99 ± 0.10% in the winter *vs* 3.72 ± 0.09; 3.78 ± 0.09 and 3.50 ± 0.08% in the summer. In multiparous groups, levels of milk fat contents for the control, HSLF and HSLF-HFLS treatments were respectively 3.78 ± 0.05; 3.92 ± 0.08 and 3.85 ± 0.06% in the winter *vs* 3.78 ± 0.05; 3.65 ± 0.06 and 3.70 ± 0.07% in the summer. This finding was in agreement to reports by Kadzere *et al*. (2002), stating declines in milk fat contents to be a direct negative effect of high environmental temperatures in the secretory function of the udder.

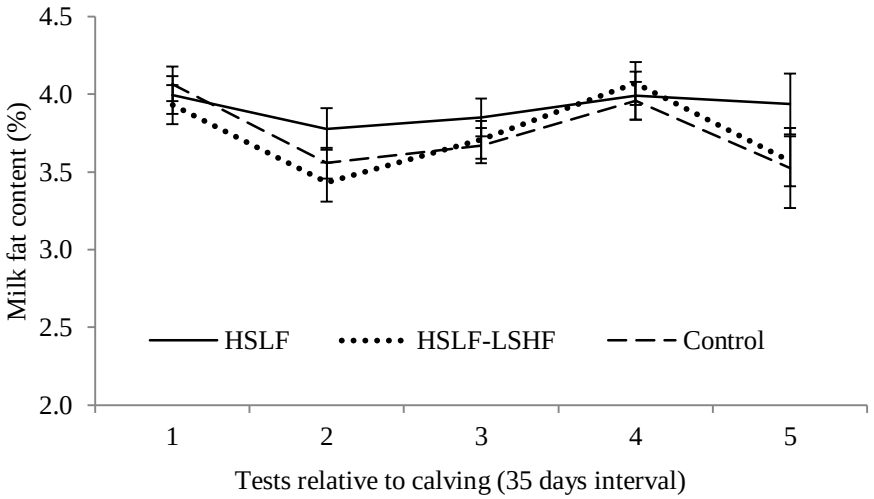

Figure 4.7 The effect of nutritional treatments differing after calving in energy levels and sources on the milk fat content (mean ± SE) recorded for primiparous dairy cows at a 35 days interval during a 154 day postpartum period.

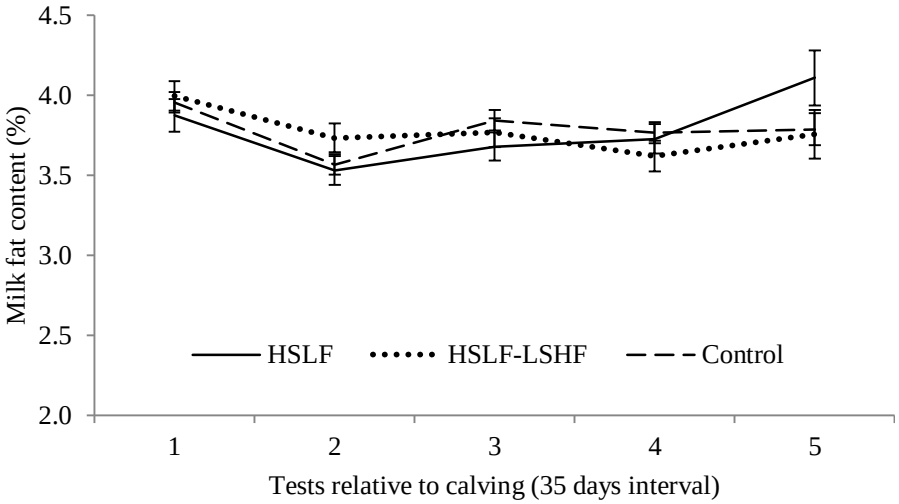

Figure 4.8 The effect of nutritional treatments differing after calving in energy levels and sources on the milk fat content (mean ± SE) recorded for multiparous dairy cows at a 35 days interval during a 154 day postpartum period.

In this study, milk fat concentrations in both primiparous (Table 4.1 and Figure 4.7) and multiparous cows (Table 4.1 and Figure 4.8) were similar between the nutritional treatments and ranged from 3.74 to 3.91%. In addition, no effects of interactions between treatments and time of observations were recorded regarding fat milk percentage for both the primiparous and multiparous cows. This finding was in agreement with Hoffman *et al*. (1993), who suggested that adequate fibre levels were consumed from pastures and the concentrate supplements, so that milk fat content was maintained in both nutritional treatments. Some studies found that milk fat content was usually enhanced after feeding lipogenic diets and decreased when feeding glucogenic diets (Schroeder *et al*., 2004; Van Knegsel *et al*., 2005, 2007a, b; Garnsworthy *et al*., 2008a, b, 2009; Reis *et al*., 2012), while others found no effects (Dyck *et al*., 2011; Gilmore *et al*., 2011). Other reports indicated that adding either high dietary starch or fat levels into diets can lead to a depression in milk fat yield in dairy cows. Feeding insulinogenic or glucogenic diets to dairy cows can promote glucogenesis over lipogenesis due to the low availability of fat precursors, to subsequently reduce fat synthesis in the udder and milk energy output (Van Knegsel *et al*., 2007a). Another report established that the decrease in milk fat is probably due to a build-up of trans fatty acids in the rumen, because of the low pH with high starch diets (Kalscheur *et al*., 1997). Bauman and Griinari (2001) argued that the reduction in milk fat percentage when overfeeding fat is generally related to altered rumen function, fat biohydrogenation and ruminal formation of trans-10 C18:1 fatty acids. It was reported that an increased supply of trans-10 cis-12 conjugated linoleic acid over other fatty acids to the udder to be responsible for depression in milk fat content in dairy cows (Gama *et al*., 2008). This fatty acid has

been recognized as a possible inhibitor of milk fat synthesis, decreasing the activity of lipogenic enzymes in the udder (Baumgard *et al*., 2002).

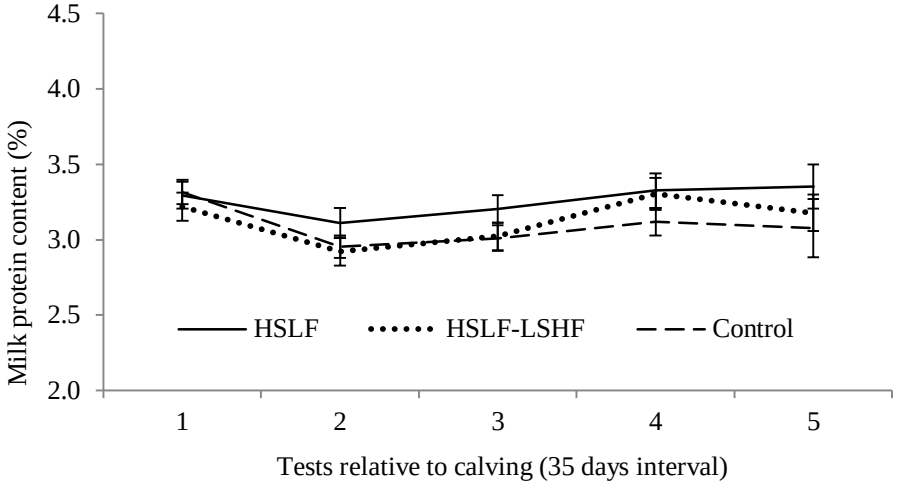

Figure 4.9 The effect of nutritional treatments differing after calving in energy levels and sources on the milk protein content (mean ± SE) recorded for primiparous dairy cows at a 35 days interval during a 154 day postpartum period.

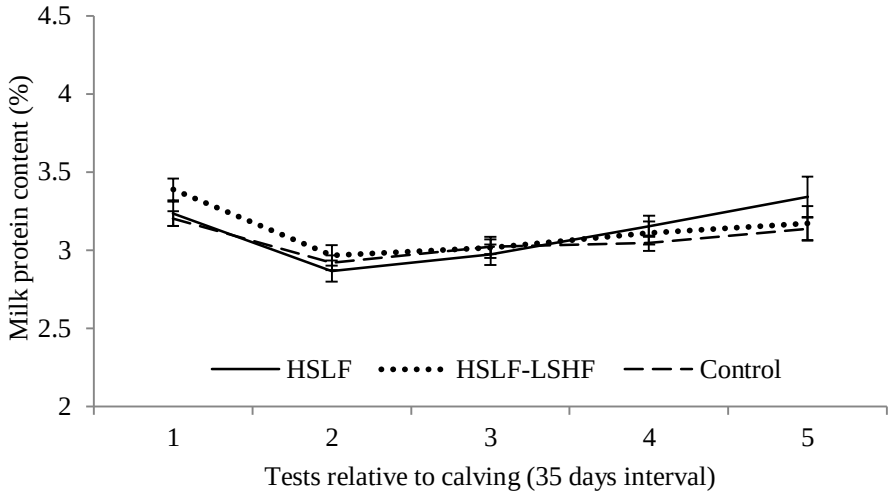

Figure 4.10 The effect of nutritional treatments differing after calving in energy levels and sources on the milk protein content (mean ± SE) recorded for multiparous dairy cows at a 35 days interval during a 154 day postpartum period.

The effects of the energy level and sources of supplements on milk protein contents are presented in Figure 4.9 for primiparous and 4.10 for multiparous cows, respectively. The calving season effect and the interaction effect between calving season and treatment did not affect the milk protein content of both primiparous and multiparous cows. This present finding was in contrast to reports by Kadzere *et al*. (2002) stating declines in milk protein percentage to be a result of heat stress on

dairy cows in the hot climatic seasons. However, primiparous cows receiving the HSLF treatment recorded a high milk protein content (*P = 0.04*), compared to their counterparts in the HSLF-LSHF and the control (i.e. 3.26, 3.10 *vs* 3.13%; Table 4.1 and Figure 4.9) while no effects were recorded within multiparous cows (Table 4.1 and Figure 4.10). No interaction effects between nutritional treatment and time of observation were detected regarding milk protein content of both the primiparous and multiparous groups. Milk protein percentage increased when feeding glucogenic diets (Voigt *et al*., 2003), but decreased with lipogenic diets (Erickson *et al*., 1992; Harrison *et al*., 1995; Chouinard *et al*., 1997). However, other reports showed no effects on milk protein content when feeding either starch- or fat-based diets or their combinations (Garnsworthy *et al*., 2009; Dyck *et al*., 2011; Gilmore *et al*., 2011). The enhanced protein levels are likely to be explained by contributory factors of increased glucose availability with starch based diets. These diets increased plasma glucose availability to the udder (Piccioli-Cappelli *et al*., 2014), sparing of the absorbed amino acids in the small intestine from hepatic gluconeogenesis (Granzin, 2004). Although not measured in the present experiment, increased glucose and insulin, that are respectively important energy metabolite and hormone in the metabolic partitioning of nutrients, increased milk protein yield (Mackle *et al*., 1999) and decrease milk fat yield (Gaynor *et al*., 1995; Reynolds *et al*., 2001). Higher protein content when the HSLF treatment was fed to primiparous cows could be attributed to a higher plasma insulin concentration (McGuire *et al*., 1995; Van Knegsel *et al*., 2007b) and a greater uptake of amino acids by the mammary gland (Hills *et al*., 2015). In contrast, Garnsworthy (1997, 2002) suggested that a deficiency in plasma glucose, induced by either an increase intestinal absorption of free fatty acids or a higher synthesis of lactose, reduced milk protein percentage in dairy cows. A possible deficit in plasma glucose of dairy cows in the control and HSLF-LSHF treatment may partly explain the reduction of milk protein percentage in comparison to HSLF treatment in the current study. Further reports also argued that dietary fat supplementation actually may be a strategy to limit rumen microbial synthesis and glucogenesis, leading to a poor protein synthesis in the udder (Palmquist, 1988). Another possible explanation was that supplementation with bypass fat can lower milk protein yield via the negative effects on blood insulin and glucose concentrations (Hills *et al*., 2015). Furthermore, the variation in responses of the milk composition with energy supplementation can possibly be related the amount and composition of lipogenic (chain length, type and degree of saturation of long chain fatty acids) (Van Knegsel *et al*., 2007a) and glucogenic nutrients (rate of fermentation in the rumen) (Hills *et al*., 2015). These physico-chemical characteristics have been shown to affect the profile of the nutrients absorbed from the GIT into the bloodstream (Reynolds *et al*., 1994) which in turn may affect the animal energy

metabolism (Williams & Stanko, 2000; Van Knegsel *et al*., 2007b, c; Garnsworthy *et al*., 2009) and influence the milk production and milk composition (Reynolds *et al*., 1988; Van Knegsel *et al*., 2005; Reis *et al*., 2012; Higgs *et al*., 2013).

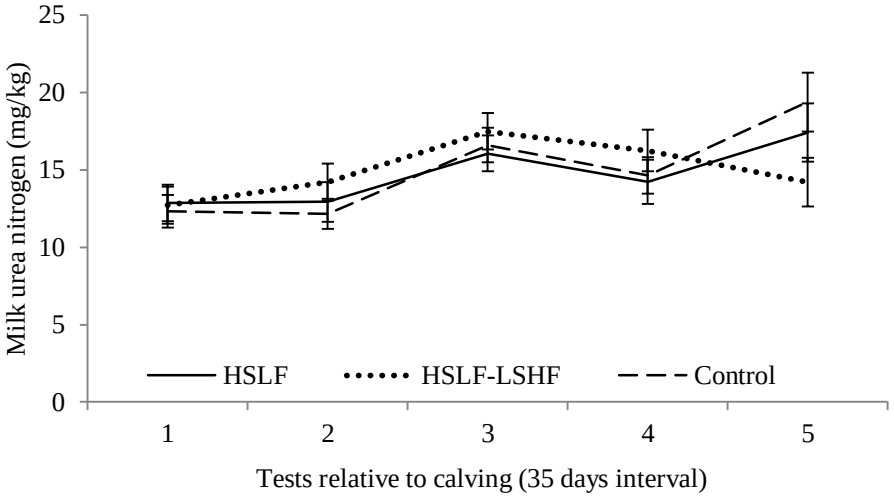

Figure 4.11 The effect of nutritional treatments differing after calving in energy levels and sources on the milk urea nitrogen (MUN) levels (mean ± SE) recorded for primiparous dairy cows at a 35 days interval during a 154 day postpartum period.

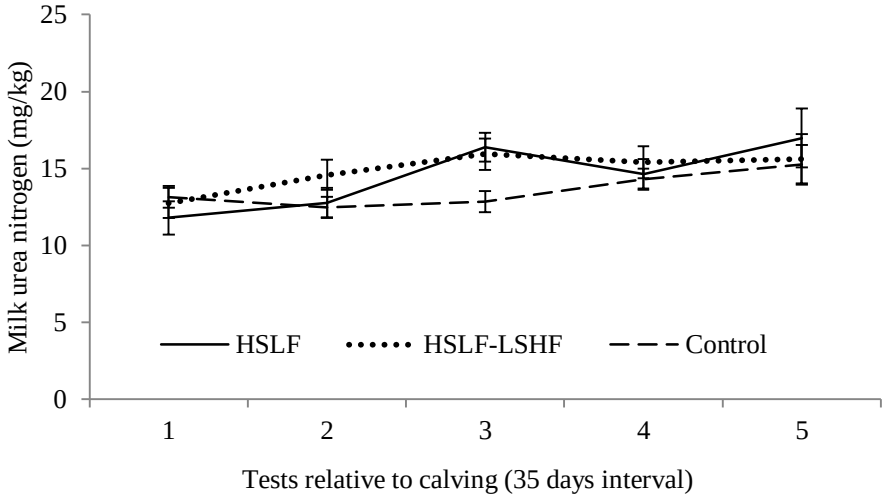

Figure 4.12 The effect of nutritional treatments differing after calving in energy levels and sources on the milk urea nitrogen (MUN) levels (mean ± SE) recorded for multiparous dairy cows at a 35 days interval during a 154 day postpartum period.

The effects of the energy type and level of supplements on MUN are illustrated in Figure 4.11 for primiparous and Figure 4.12 for multiparous cows, respectively. In both parity groups, levels of MUN were higher ($P < 0.05$) in the winter, compared to the summer season. No interaction effect between calving season and treatment was recorded regarding MUN for both primiparous and

multiparous cows. In the primiparous groups, levels of MUN for the control, HSLF and HSLF-HFLS treatments were 15.9 ± 0.81; 16.1 ± 0.91 and 16.0 ± 0.94 mg/kg, respectively, in the winter *vs* 13.9 ± 0.88; 13.8 ± 0.82 and 14.2 ± 0.74 mg/kg in the summer. In the multiparous groups, levels of MUN for the control, HSLF and HSLF-HFLS treatments were 14.6 ± 0.50; 15.8 ± 0.60 and 15.8 ± 0.64 mg/kg, respectively, in the winter *vs* 12.7 ± 0.48; 13.0 ± 0.59 and 14.0 ± 0.74 mg/kg in the summer. The seasonal variations in MUN in the present study can be attributed to variations in milk content of dairy cows as result of changes in seasons of calving (Mostert *et al*., 2001; Kadzere *et al*., 2002). However, levels of MUN in the current study were similar between nutritional treatments in both primiparous (Table 4.1 and Figure 4.11) and multiparous cows (Table 4.1 and Figure 4.12). In addition, no effects on interaction effect between treatment and time of observations were recorded regarding the MUN levels.

The MUN content is indicative of the synchronisation between energy and protein availability in the rumen, which is important for maximum production of microbial protein (Huntington, 1997). Broderick and Clayton (1997) reported that MUN concentration serves as an index of the inefficient utilization of dietary protein in lactating dairy cows. A poor balance between rumen degradable protein and fermentable carbohydrate results in decreased N utilisation in the rumen and increased absorption of ammonia, which is subsequently converted to urea in the liver and secreted in milk (Moharrery, 2004). It has been indicated that MUN concentrations above 14 mg/kg of milk suggest an insufficient supply of fermentable energy per unit of CP (especially degradable CP) or ingestion of CP in excess of dietary needs (Broderick & Clayton, 1997). Although not significant in this study, MUN levels ranged from 13.7 to 15.1 mg/kg, suggesting a poor N utilisation in the rumen. Based on these values, one possible explanation for MUN levels was obviously related to the increase in plasma urea [observed in Chapter 3 (3.3)], which was elucidated by the changes in the structural properties of the pelleted concentrates to rumen fermentation. The heat treatment in the pelleting process possibly have increased its resistance to the rumen fermentation and/or rapidly bypassed the energy nutrients to the small intestine, restricting the N capture for MPS in the rumen. This limitation resulted in the increased absorption of ammonia, which is subsequently converted to urea in the liver and secreted in milk.

4.4. Conclusion

In this study, feeding high levels of concentrates (i.e. 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively) to dairy cows in HSLF and HSLF-LSHF treatments significantly increased milk yield, compared to those fed the lower concentrate level (i.e. 7 kg/day for both

primiparous and multiparous cows) in the control in a pasture based system. This response indicated an increase in the total nutrient intake, favouring improvements in cows of the digested nutrients, ME, and somatotropic axis for high milk production in HSLF and HSLF-LSHF treatments in comparison to the control. In the primiparous group, the HSLF diet enhanced milk protein content whereas HSLF and HSLF-HFLS diets resulted in increased milk lactose content. In multiparous group, HSLF treatment increased milk lactose content. Different inclusion levels and sources of energy nutrients on the somatotropic axis, that sustain not only the milk synthesis but also a rapid restoration of body reserves and an early fertility success (i.e. postpartum health, uterine regression, resumption of oestrous cycles, conception and embryo/pregnancy survival), warrant further investigation in dairy cows. Also, other energy sources (i.e. oat, barley, wheat, canola and rapeseed) and their combinations on milk responses of dairy cows grazing on other irrigated forages (i.e. cynodon, digitaria, paspalum, etc.) produced in Western Cape warrant further investigation.

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Chapter 5

Effect of energy sources on ovarian follicular dynamics and oestrous activity of Holstein cows

Abstract

The objective of the study was to evaluate the effect of nutritional treatments differing in energy levels and sources on ovarian follicular dynamics and oestrous activity in dairy cows. Twenty two Holstein multiparous cows from the Elsenburg herd were used in this study. After parturition, cows were maintained on kikuyu-ryegrass pastures on *ad libitum* basis and allocated to different levels and types of concentrate supplements differing in starch and/or fat contents. The control received 7 kg/day of a control concentrate while the treatment groups received each 12.6 kg/day of concentrate. The concentrates in treatments contained high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) levels. The supplement in treatment HSLF was a glucogenic concentrate using maize as the energy source. The supplements in treatment HSLF-LSHF were a combination of a glucogenic concentrate offered for the first 60 days in milk (DIM) like in treatment HSLF, followed from 61 DIM by a lipogenic concentrate using wheat bran and calcium (Ca)-salts of long-chain fatty acids as the energy sources. At 80 ± 10 DIM, cows were synchronised using an Ovsynch protocol without being inseminated before the ultrasonography observation. While being held in a shaded neck clamp, cows were assessed individually using an ultrasound scanner every three days for ovarian measurements and follicular activity until the subsequent oestrus. Results showed that ovarian and follicular measurements as well as numbers of follicles within different follicle size classes were similar between nutritional treatments. The total ovarian follicular counts were higher ($P < 0.05$) in cows receiving the HSLF and HSLF-LSHF treatments, compared to their counterparts in the control group (i.e. 7.23 ± 0.22, 7.21 ± 0.14 *vs* 6.53 ± 0.19, respectively), through an improvement in nutritional status. Further research is required to investigate different energy levels and sources that enhance the viability and the quality of the oocyte ovulating from the dominant follicle and improve the intensity and length of the oestrous expression in dairy cows.

5.1. Introduction

Optimizing the reproductive efficiency in dairy herds requires cows to calve annually to maximize the economic returns from milk production (Roche *et al.*, 2000; Lucy, 2001). However, several studies have reported a worldwide decline in the fertility of dairy cows (Lucy, 2001, 2007; Butler,

2003; Walsh *et al*., 2011). Current evidence has established that the peripartum NEB can last in dairy cows up to 10 to12 weeks postpartum (Butler, 2003) and suppresses the ovarian axis (Chagas *et al*., 2007; Santos *et al*., 2008; Evans & Walsh, 2012), resulting in extended anoestrous periods (Butler, 2000). The anoestrus occurs through attenuation of the frequency of the luteinizing hormone (LH) pulse and low levels of plasma glucose, insulin and insulin-like growth factor-I (IGF-I), collectively preventing oestrogen production by dominant ovarian follicles (Beam & Butler, 1999; Diskin *et al*., 2007). This condition indicated that a NEB has an adverse effect on the follicular and consequent luteal development (Wathes *et al*., 2007) and also on the quality of the ovulated oocyte, suggesting possible long term carry-over effects on fertility through impairment in the developmental competence of oocytes (Britt, 1994). Consistent with this argument, several reports indicated that the ovarian dysfunction and/or reduced oocyte competence play an important role in reducing fertility of dairy cows (Lucy, 2001, 2007; Leroy *et al*., 2008a, b, 2014).

In this regard, the ultrasound imaging technique presents a way to study bovine follicular dynamics in order to understand the ovarian function on fertility in dairy cows (Pierson & Ginther, 1987; Savio *et al*., 1988; Sirois & Fortune, 1988; Sakaguchi *et al*., 2004; Mossa *et al*., 2012). This technique was initially used during the normal oestrous cycle as an aid to develop effective oestrous synchronization strategies (Ireland *et al*., 2000). Real time ultrasonographic imaging allows non-invasive, visual assessment of changes in ovarian structures over a period of time (Fricke, 2002). Furthermore, this procedure is convenient, since it is an easy and fast tool to use at the farm level, without affecting management routines in dairy cows (Silva-Santos *et al*., 2013). Little is still known in *in vivo* systems of the effect of energy levels and sources on folliculogenesis and oestrous activity in dairy cows. Therefore, the objective of the current study was to evaluate the effect of inclusion level (low *vs* high) and type of energy supplements (starch *vs* fat) on ovarian follicular dynamics and oestrous activity in grazing Holstein cows following oestrous synchronisation.

5.2. Materials and Methods

The ethical clearance for this study was obtained from the Western Cape Department of Agriculture (WCDA, Project AP/BR/D/CM31).

5.2.1. Experimental location

The trial was conducted at the Elsenburg Research Farm (WCDA), as described in Chapter 3 (3.2.1).

5.2.2. Experimental husbandry, animals and diets

The current trial was simultaneously conducted with the preceding trial described in Chapter 3 (3.2.2) with the same feeding management, i.e. control, HSLF and HSLF-LSHF supplements fed to grazing Holstein cows on kikuyu-ryegrass pastures. The ovarian follicular dynamics and oestrous activity of 22 multiparous Holstein cows (i.e. of 3.89 ± 0.40 years of age in their second and third parities) were assessed over an oestrous period (± 21 days). Following parturition, cows were allocated into three isonitrogenous nutritional treatments, according to the calving date, LW at calving and milk yields during the previous lactation. The nutritional treatments consisted of *ad libitum* access to cultivated irrigated kikuyu-ryegrass pastures, associated with different levels and types of concentrate supplementation (Table 3.1 in Chapter 3). The control group received 7 kg/day of a control concentrate supplement, while concentrate supplements in treatment groups were fed at 12.6 kg/day. Half of the concentrate allowance was fed after each milking and cows were milked twice a day at 05:30 and 15:00. The control diet contained low level of energy content (2.47 Mcal ME/kg DM) and provided 457, 104 and 40 g/kg of NDF, starch and fat on DM basis, respectively. The supplement in HSLF treatment was a glucogenic concentrate containing maize as the energy source. The HSLF diet contained high level of energy content (2.71 Mcal ME/kg DM) and offered 341, 242 and 35 g/kg of NDF, starch and fat on DM basis respectively. This HSLF diet was formulated to increase the circulating glucose and insulin levels in order to improve the postpartum EB status and encourage ovarian activity in cows. The supplements in the HSLF-LSHF treatment were combinations of a glucogenic concentrate offered for the first 60 DIM per treatment HSLF, followed from 61 by a lipogenic (LSHF) concentrate using wheat bran and Ca-salts of long-chain fatty acids (Megalac rumen bypass fat, Volac International Ltd., UK) as the energy sources. In the HSLF-LSHF combination treatment, the high starch-based diet was fed to achieve the same objective of the HSLF treatment during the first 60 DIM. The LSHF diet contained high level of energy content (2.70 Mcal ME/kg DM) and was formulated to provide 388, 137 and 58 g/kg of NDF, starch and fat on DM basis, respectively. Secondly, the LSHF diet was fed from 61 DIM to decrease plasma insulin and improve plasma cholesterol in order to increase the ovulatory follicular size.

5.2.3. Experimental sampling and data collection

At 80 ± 10 (Standard deviation, SD) DIM, 22 cows were synchronised using an Ovsynch protocol without a subsequent insemination. Cows were handled individually with care, while being restrained in a sheltered neck clamp. The synchronisation procedure started from early August 2014

and was as follows: (i.) At 11:00 on d 0, cows received an intravaginal progesterone (P_4) device (Controlled internal drug release, CIDR, Pfizer Laboratories Pty Ltd, Sandton, South Africa) containing 1.9 g P_4 together with an intramuscular injection of 2 mL of the gonadotropin releasing hormone (GnRH) (Cidirol containing oestradiol benzoate 1 mg/mL benzyl alcohol 100 mg/mL as preservative; Pfizer Laboratories Pty Ltd, Sandton, South Africa); (ii.) On d 7, animals received an intramuscular injection of 1 mL prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$, Estrumate containing Chorocresol 0.1% m/v as preservative and Cloprostenol sodium 263 µg, equivalent to 250 µg Cloprostenol, Schering-Plough Animal Health, Isando, South Africa) at 16:00; (iii.) On d 8, CIDR devices were removed at 07:00 and heat detectors (Kamar® Heatmount Detectors, Kamar Products Inc., Zionsville, USA) were attached to cows to the tail-head area of cows; (iv.) On d 9, a dose of 1 mL of $PGF_{2\alpha}$ was administrated by an intramuscular injection; and (v.) On d 10, cows were not inseminated, but positive heat detectors were removed and the ovaries were subjected every three days to an ultrasonographic evaluation for the next 21 ± 3 days until the subsequent oestrus. To avoid any ultrasonographic procedure on Sundays, an interval of four days was sometimes used. The diagnostic imaging was performed using an ultrasound scanner (SonoScape A6, SonoScape Medical Corp., Shenzhen, China), with a linear transrectal probe and standardised settings. Settings were 5-6 MHz for the frequency; 80% for power and 62mm for depth. This procedure was conducted by a single trained veterinarian for all cows to minimise variation. The Kamar detectors were attached to cows on d 9 from the start of the ultrasound observation, and cows were artificially inseminated when the oestrous detection was positive. The Kamar detector was positive when cows were showing typical oestrous behaviours (i.e. mucous vaginal discharge, mounting, sniffing, etc.). The trial was completed following a pregnancy diagnosis by rectal palpation by the veterinarian at 35-40 days after artificial insemination (AI).

Ovarian follicles, observed as black circular fluid filled structures, were identified on all digitized images (Fricke, 2002). An electronic calliper was used to measure the diameters of the ovaries and follicles. The relationship between the measured dimensions on the digitized images and the calculated dimensions on the ultrasound were determined using the established linear regression ($y = 0.392\ x$, $R^2 = 0.84$). All visible follicles were counted on both ovaries following each ultrasound observation and the total number of follicles (for both ovaries) per animal was noted, as were the areas (eclipses) of the ovaries over time. The area of the eclipse was measured as Area = 3.142 x Short radius x Long radius, with short and long radiuses being half of their respective diameters. Follicles were categorised in relation to the long diameter in size classes according to Garnsworthy

et al. (2008c) as follows: small sized follicles being < 5 mm in diameter, medium sized follicles 5 to 10 mm in diameter and large sized follicles > 10 mm in diameter.

5.2.4. Statistical analysis

Data were analysed using the PROC MIXED of SAS enterprise guide (SAS, 2012). The statistical model included the treatment (*T*) effect, time (*t*) effect of observations and interaction effect between treatment and time (*Tt*) as fixed effects, while animal effect within treatments was specified as a random effect. The measured variables obtained within an ultrasound observation during the trial were considered as repeated observations of a particular block [chapter 3 (3.2.4) for justification]. The statistical model was defined as follow:

$Model = \mu + T_i + t_j + (tT)_{ij} + \delta_{(ij)k} + \varepsilon_{ijk}$, where

μ = overall mean;

T_i = the fixed effect of the i^{th} treatment (i.e. Control, HSLF and HSLF-LSHF);

t_j = the fixed effect of the j^{th} test of ultrasound observation (i.e.1 to 7);

$(tT)_{ij}$ = the interaction between levels i^{th} treatment and j^{th} test of ultrasound observation;

$\delta_{(ij)k}$ = the variable effect of the k^{th} block effect in the i^{th} treatment (Repeated statement);

ε_{ijk} = the random experimental error.

All effects were used to analyse the ovarian dimensions whereas the follicular counts were analysed without the repeated statement ($\delta_{(ij)k}$) in the statistical model. The area of the preovulatory follicle at the first ultrasound observation was analysed without the repeated statement ($\delta_{(ij)k}$) and all interactions using the treatment as the only fixed effect in the statistical model. Statistical assumptions were described as fixed effects and their interactions were equal to zero with $\delta_{(ij)k} \sim N(0, \sigma_e^2)$, varying independently of ε_{ijk}. Differences in means and standard error (SE) of means between treatments were obtained using the pair wise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

5.3. Results and Discussion

Table 5.1 presents the effect of concentrate supplementation differing in energy levels and types in a pasture-based system on ovarian and follicular dimensions, follicular counts and proportions of cows ovulating and pregnant. The changes in total ovarian follicles of dairy cows over time of observation until the subsequent oestrus are illustrated in Figure 5.1.

Table 5.1 The effect of nutritional treatments differing after calving in energy levels and sources on ovarian and follicular dimensions, follicular counts (mean ± SE) and proportions of Holstein cows ovulating and pregnant following an Ovsynch protocol recorded during a 21 ± 3 day period.

	Concentrate feeding			*P*-values		
Parameters	**Control**	**HSLF**[1]	**HSLF-LSHF**[1]	T^2	t^2	Tt^2
Number of cows	10	6	6			
Ovarian and follicular dimensions						
Short diameter of the ovary (mm)	16.2 ± 0.42	16.3 ± 0.43	16.1 ± 0.32	0.22	0.76	NS
Long diameter of the ovary (mm)	33.1 ± 0.62	33.4 ± 0.79	33.7 ± 0.81	0.32	0.87	NS
Ovarian area (mm^2)	426 ± 15	437 ± 19	445 ± 20	0.29	0.73	NS
Area of preovulatory follicles at first observation (mm^2)	115 ± 15	167 ± 21	131 ± 19	0.16	-	-
Follicular counts						
Number of small follicles	3.16 ± 0.36	3.46 ± 0.42	3.58 ± 0.30	0.19	0.09	NS
Number of medium follicles	2.04 ± 0.17	2.48 ± 0.22	2.16 ± 0.24	0.42	0.08	NS
Number of large follicles	1.30 ± 0.08	1.29 ± 0.11	1.44 ± 0.11	0.58	0.07	NS
Number of total follicles	6.53^a ± 0.19	7.23^b ± 0.22	7.21^b ± 0.14	0.03	0.09	NS
Proportion of cows ovulating and pregnant[3]						
Proportion of cows ovulating after Ovsynch	1.00	0.83	1.00	-	-	-
Proportion of cows showing oestrous expression	0.50	0.66	0.83	-	-	-
Pregnancy rate after artificial insemination	0.40	0.50	0.50	-	-	-

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat. [2]*T*: Treatment, *t*: test of ultrasound observation, *Tt*: Interaction Tt.

[3]Not statistically analysed due to the small data set (n=22).

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

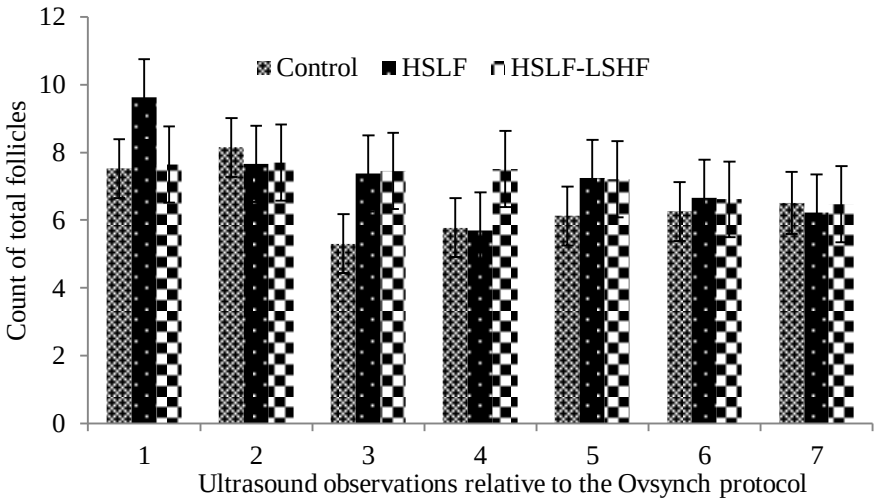

Figure 5.1 The effect of nutritional treatments differing after calving in energy levels and sources on the total number of ovarian follicles (mean ± SE) following an Ovsynch protocol recorded during a 21 ± 3 day period.

The mean (± SE) area of the bovine ovaries between nutritional treatments was 436 ± 18 mm^2, with a long diameter of 33.5 ± 0.74 mm and a short diameter of 16.2 ± 0.39 mm. The ovarian dimensions

of grazing Holstein cows were similar between nutritional groups differing in inclusion level and type of concentrate supplements. Also, no interactions between the treatment and the time of observation were detected. Furthermore, the areas of the preovulatory follicle, considered as the largest follicle at first ultrasound observation of cows, were similar between nutritional treatments. Also, the energy levels and types between nutritional treatments did not affect the number of follicles within the different follicle size classes of dairy cows. However, cows receiving 12.6 kg/cow per day of concentrates in HSLF and HSLF-LSHF treatments increased ($P < 0.05$) the number of total follicles, both amounting to 15% more follicles, in comparison to cows receiving only 7 kg/cow per day of concentrate in the control.

Although the molecular mechanisms involved in the acquisition of competence are not well known, the follicle size is an important parameter that influences oocyte competence (Blondin *et al.*, 2012). Lonergan *et al*. (1994) and Blondin and Sirard (1995) studied *in vitro* the effect of follicular size on the quality and viability of oocytes in dairy cows and found that oocytes emerging from smaller follicles (<3 mm) have reduced or no developmental competence. These researchers reported that these oocytes appear to have been recovered too early and therefore probably lacked some follicular factors that would have signalled the oocytes to acquire their full competence. These factors, consisting of maternal messenger RNA and protein molecules, are synthesized and/or stored in a time, space and dose dependent manner in the nucleus and ooplasma during the oocyte growth and maturation (Blondin *et al.*, 2012). Barnes *et al.* (1993) reported that an oocyte ovulating from a larger dominant follicle has higher developmental competence in terms of quality and viability. This competence is the ability of the oocyte to complete maturation, undergo successful fertilisation, reach the blastocyst stage and yield a healthy embryo (Watson, 2007). Using an *in vivo* system, Vasconcelos *et al*. (2001) studied the effect of follicle aspiration and GnRH treatment on the size of follicles and corpus luteum of lactating dairy cows. These researchers reported that increased follicle sizes in non-aspirated cows have advantageous effects on the corpus luteum function and its secretion of oestrogen and P_4, compared to the aspirated group. No differences in the preovulatory follicle diameter (Area = 3.412 x R^2, with R being the radius) were recorded when isocaloric diets, differing in increasing levels of either starch or fat, were fed to lactating Holstein dairy cows (Garnsworthy *et al.*, 2008b, c). In the current study, the areas of the preovulatory follicle were 45% and 14% larger in lactating dairy cows fed HSLF and HSLF-LSHF treatments compared to those fed the control diet, respectively. However, the recorded difference was not significant ($P = 0.16$), possibly due to the small number of cows used in the study (n=22).

Several studies investigated the effect of nutrition on ovarian axis and reported that diets have a direct effect on the ovarian activity, affecting the follicular development and oocyte competence (Garnsworthy & Webb, 1999; Garnsworthy *et al*., 2008a; Bisinotto *et al*., 2012). Similarly, other studies noted that the level of energy intake can affect circulating levels of steroids and growth factors, all together stimulating follicular dynamics (O'Callaghan & Boland, 1999; Webb *et al.* 2004). Adamiak *et al.* (2005) studied the follicular growth in 20 months old beef x dairy heifers fed either 500kj or 1000 KJ ME/kg of metabolic LW per day. These researchers found no effect on the total number of visible follicles, but an increased number of medium-sized (4-8 mm) and large (>8 mm) follicles as well as improved growth rate and maximum diameter of the dominant follicles when feeding the high metabolizable energy (ME) diet to compared to the low ME diet. On the other hand, Gong *et al.* (2002a) studied the ovarian follicular responses to follicle stimulating-hormone (FSH) treatment in Hereford x Friesland heifers fed on DM basis 2 kg of hay + 2.5 kg of concentrates at maintenance level and 2 kg of hay + 6.5 kg of concentrates at twice maintenance level. These researchers established that feeding heifers at twice the maintenance level resulted in a significant increase in the number of small (2-4 mm) and large (>9 mm) follicles. Recently, Mossa *et al*. (2012) reported that low follicular counts of ≥ 3 mm in diameter were associated *in vivo* with lower probabilities of fertility in dairy cows. The decline in number of ovarian follicles was reported to be associated with small ovaries and lower circulating P_4 levels (Ireland *et al*., 2008), as well as reduced endometrial development of animals (Jimenez-Krassel *et al*., 2009).

A state of NEB in postpartum dairy cows compromises the somatotropic axis and disrupts the influence of the gonadotropins on their ovary, preventing the dominant follicle from ovulating (Beam & Butler, 1999). In other words, a severe NEB during postpartum period suppresses the pulsatile LH secretion, reduces oestradiol levels and decreases the ovarian responsiveness to LH stimulation in dairy cows (Butler, 2000; Diskin *et al.*, 2007). The decline in these hormones decreases the diameter and growth rate of the dominant follicle as well as the weight of the corpus luteum (Beam & Butler, 1997, 1999) and eventually results in delayed ovulation (Butler, 2003). Furthermore, Roche *et al*. (2000) also reported that postpartum anovulatory oestrus in dairy cows is not due to an absence of follicular development, but rather the failure of a dominant follicle to ovulate, because of a NEB. Also, the ovaries that exposed to adverse conditions of the NEB were reported to be less capable of producing adequate amounts of oestrogens and P_4 after ovulation (Leroy *et al*., 2008a). Wathes *et al*. (2007) reported that inferior oocytes were produced in dairy cows at the start of breeding season when ovarian follicles underwent their earlier stages of maturation during the NEB nadir. Consistent with these arguments, other studies where dairy cows

were monitored using milk P_4 profiles have reported that the NEB is associated with a greater incidence of irregular oestrous cycles, resulting in poor fertility outcomes (Taylor *et al*., 2003; Wathes *et al*., 2003). Lucy *et al.* (1991a) found that the EB status in early lactation influenced early changes in follicular populations of lactating dairy cows and reported decreasing numbers of small (< 5 mm) ovarian follicles and increasing numbers of large (> 8 mm) follicles, with increasing days postpartum. Also, lactating dairy cows supplemented with lipids in early lactation in the attempt to improve the EB status were found to have follicles with larger diameters (Lucy *et al*., 1991b). Beam and Butler (1997) studied the influence of EB status and level of dietary fat on the dominant follicle development and function in lactating Holstein cows. These researchers found that follicles emerging after the NEB nadir rather than before showed improved follicular growth and diameter, enhanced oestradiol production, and were more likely to participate in ovulation.

Although insulin and IGF-I levels were not measured in this study, these hormones are signals mediating the critical changes in EB status. Such hormonal improvements in dairy cows support the postpartum number, differentiation and maturation of follicles (Roche *et al*., 2011), thereby increasing the oestradiol production (Gong *et al*., 2002b) and the chance of dominant follicles to ovulate in response to the LH surge (Beam & Butler, 1997, 1999; Lucy, 2003). Other reports argued that the NEB which is a physiological state of undernutrition has been related to the inability of the pituitary gland to sustain high frequency LH pulses to the ovaries, resulting in a prevention of an ovulation taking place (Schillo, 1992; Jolly *et al*., 1995). The postpartum frequency of LH pulses was found to be significantly lower during the first follicular wave in cows that developed a non-ovulatory dominant follicle, compared to those in which the dominant follicle continued to develop and eventually ovulate (Beam & Butler, 1999). This observation demonstrated the dependence of the ovulation of the dominant follicle on the re-establishment of the LH surge, encouraging preovulatory growth and oestradiol secretion (Butler, 2003). Several reports indicated that energy sources can be manipulated via ingredients in the diet to prevent and/or treat the NEB of postpartum dairy cows (Staples *et al*., 1998; Gong *et al*., 2002b; Jorritsma *et al*., 2003; Van Knegsel *et al*., 2005, 2007a, b, c, d; Gilmore *et al*., 2011; Thatcher *et al*., 2011). Increasing the supply of glucogenic contents in the diet of dairy cows exerts a stimulating effect at the ovarian level (Letelier *et al*., 2008), encouraging folliculogenesis (Scaramuzzi *et al*., 2011). However, excessive insulin and IGF-I levels from high starch-based diets may over-stimulate the ovarian developmental competence of oocytes and thus yield inferior oocytes in dairy cows (Armstrong *et al*., 2001; Fouladi-Nashta *et al*., 2005; Laskowski *et al*., 2016). The decrease in quality and viability of oocytes occurs as the synthesis and accumulation of maternal messenger RNA and protein

molecules are uncoupled in an insulinogenic condition during the oocyte growth and maturation (Leroy *et al*., 2008b). In contrast, feeding high fat diets after a voluntary waiting period, like in HSLF-LSHF treatment, increases the number and size of follicles, as well as the oestradiol secretion of the preovulatory follicle (Lucy *et al.*, 1991b; Beam & Butler, 1997; Moallem *et al*., 2007), most likely via the induction of high cholesterol levels in the follicular fluid and plasma (Van Knegsel *et al*., 2007a). In the present pasture-based study, HSLF and HSLF-LSHF treatments were designed to provide isocaloric concentrates differing in starch and fat contents at an inclusion level of 12.6 kg/day, compared to 7 kg/day of concentrate in the control group. The total number of ovarian follicles (Figure 5.1) showed a positive relationship with the inclusion levels (high *vs* low) of concentrates, indicating an increased ovarian follicular growth in dairy cows. Such improvements possibly suggested improvements of specific receptors, metabolites and hormones signalling the follicular growth in dairy cows. This positive effect in the current study can be attributed to the levels of concentrate supplements provided in HSLF and HSLF-LSHF treatments, improving the nutritional status (i.e. total digested nutrients and ME) in comparison to the control. This nutritional improvement in HSLF and HSLF-LSHF treatments had possibly optimised the hypothalamic-pituitary-ovarian axis and the energy carry-over effect into follicular and ovarian function, compared to the control.

The proportions of cows showing oestrous expression in the subsequent oestrus were 0.50, 0.66 *vs* 0.83 for the control, HSLF and HSLF-LSHF, respectively. The proportion of cows pregnant following the AI during the subsequent oestrus were 0.40, 0.50 *vs* 0.50 for the control, HSLF and HSLF-LSHF, respectively. Although not statistically compared in the current study, several physiological events influence the expression of oestrus and pregnancy rates in dairy cows. The root cause of poor oestrous expression (poor intensity and duration) in dairy cows is related to low circulating oestradiol levels, due to its high metabolic clearance rate (Sangsritavong *et al*., 2002) and low plasma LH, insulin, IGF-I levels induced by the NEB (Diskin *et al*., 2007; Garnsworthy *et al*., 2008a) and stress (Dobson *et al*., 2008). Coupled with poor oestrous expression, an inability to easily detect oestrus can further impede the AI being performed at the correct time (Walsh *et al*., 2011). Whether the energy sources and intake levels were reported to affect fertility outcomes (Butler, 2000, 2003; Gong *et al.*, 2002a, b; Jorritsma *et al*., 2003; Garnsworthy *et al*., 2008b, c; Bisinotto *et al.*, 2012), remains to be investigated further under field conditions and a longer postpartum period (i.e. 305 DIM).

5.4. Conclusion

In this study, no differences were observed between nutritional treatments in terms of the dimensions of the ovaries and the preovulatory follicle as well as numbers of follicles within different classes in dairy cows. However, grazing cows receiving high (12.6 kg/day) levels of concentrates in the HSLF and HSLF-LSHF treatments recorded a higher number of total follicles, compared to those on the low (7 kg/day) level of concentrate in the control group. This response was related to the increase in the total nutrient intake and ME, supporting an improvement of the ovarian follicular growth in the HSLF and HSLF-LSHF treatments in comparison to the control. Future research needs to investigate the influence of different inclusion levels and sources of energy nutrients that enhance the viability and the quality of the oocyte ovulating from the dominant follicle and improve the intensity and length of the oestrous expression in dairy cows. Also, types and levels of different dietary energy nutrients on the pregnancy rates with a large number of cows under a field condition and a longer postpartum period (i.e. 305 DIM) require further investigation.

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Chapter 6

Effect of different energy levels and sources on the reproductive performance of Holstein cows

Abstract

The objective of the study was to evaluate the effect of nutritional treatments differing in energy sources and levels on the reproductive performance of Holstein cows. Sixty nine primiparous and 153 multiparous Holstein cows from the Elsenburg herd were used in the study. During the prepartum period, pregnant heifers and dry cows were maintained under similar feeding and management conditions. Following parturition, cows were allocated to three nutritional groups according to parity, live weight (LW) and milk yields during the previous lactation. Cows were maintained on cultivated irrigated kikuyu-ryegrass pastures provided *ab libitum* from calving to 154 days in milk (DIM). After each milking session, cows received concentrate supplements that differed in terms of energy levels and sources. The control group received of a control concentrate supplement fed at 7 kg/day to both the primiparous and multiparous cows. Concentrates in the treatment groups contained high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) levels. The treatment groups received 11.6 and 12.6 kg/DM of concentrates for the primiparous and multiparous cows, respectively. The HSLF supplement was a glucogenic concentrate containing maize as the main energy source. The HSLF-LSHF supplements were combinations of a glucogenic concentrate offered for the first 60 DIM per treatment HSLF, followed from 61 DIM by a lipogenic concentrate using wheat bran and calcium (Ca)-salts of long-chain fatty acids as the energy sources. Results of this study showed that the calving season did not affect fertility traits significantly. In addition, nutritional treatments in both primiparous and multiparous cows did not significantly affect some fertility traits e.g. the interval form calving to first service, the proportion of cows serviced for the first time within the first 80 DIM, the pregnancy rate at first service, the number of service per conception, the interval days open and the pregnancy rate at 100 DIM. However, the proportion of cows pregnant at 150 DIM was improved ($P < 0.05$) in cows fed HSLF and HSLF-LSHF concentrates in comparison to those receiving the control concentrate, being respectively 0.84 ± 0.09; 0.81 ± 0.10 *vs* 0.52 ± 0.12% in the primiparous and 0.76 ± 0.07; 0.81 ± 0.12 *vs* 0.56 ± 0.06% in the multiparous groups. The finding of this study showed that feeding a higher amount of concentrates had a positive effect on conception rates of grazing dairy cows. This improvement in HSLF and HSLF-LSHF treatments was related to the increased total nutrient intake and metabolizable energy (ME), which possibly acted positively on

the hypothalamic-pituitary-ovarian-uterus axis and optimised the energy carry-over effect into the fertility success, compared to the control.

6.1. Introduction

Improvements in breeding, nutrition and management have resulted in a considerable increase in milk yield in dairy cows over the past decades (Leroy *et al*., 2014). However, a prerequisite for efficient milk production throughout the lifetime of a dairy cow is the ability to calve at least once every 12 to 13 months. Fertility in dairy cows is therefore a critical factor affecting lifetime milk yield, herd average DIM, number of calves born per lifespan of a cow, calving interval and ultimately financial returns of the dairy farmer (Leroy & De Kruif, 2006; Inchaisri *et al*., 2011). A number of studies worldwide have shown a decline in reproductive performances in dairy cows (Lucy, 2001; Santos *et al*., 2009; Roche *et al*., 2011; Walsh *et al*., 2011; Leroy *et al*., 2014). In South Africa, Makgahlela (2008) showed an increase in the calving interval for dairy cows, i.e. from 386 days in 1986 to 412 days in 2004. Butler (1998) also showed more than 20% decrease in conception rate from 1951 to 1996 in a Holstein herd in the North America. Today, failure of dairy cows to conceive is considered as a key reason for culling, reducing the longevity of cows in the herd and threatening the sustainability of dairy farming (De Vries, 2006).

Different nutritional approaches to improve the reproductive efficiency of dairy cows have been studied (Jorritsma *et al*., 2003; Lucy, 2007; Van Knegsel *et al*., 2007a, b; Garnsworthy *et al*., 2008a, b, c, d, 2009; Gilmore *et al*., 2011; Esposito *et al*., 2014). Most investigations found that improving the energy balance (EB) status during the transition period plays a significant role in the subsequent fertility of dairy cows (Drackley *et al*., 2003). During the periparturient period, most cows enter a state of negative energy balance (NEB), which is associated with a number of metabolic and endocrinal changes. These changes may be detrimental, having adverse carry-over effects on the resumption and normality of the oestrous cycles and affecting the possible success of subsequent conceptions (Jorritsma *et al*. 2003; Wathes *et al*., 2007a). A prospective approach, with the aim to enhance fertility, while sustaining optimal milk yield and reducing the severity of the NEB, is to improve the postpartum energy density of the diet with dense nutrients like non-fibre carbohydrates and/or fat (Staples *et al*., 1998; Voigt *et al*., 2003; Van Knegsel *et al*., 2005; Gilmore *et al*., 2011). Starch-based diets fed to dairy cows during early lactation have been reported to improve circulating glucose and insulin levels and enhance ovarian activity (Gong, 2002; Van Knegsel *et al*., 2007a, b; Garnsworthy *et al*., 2008b). However, high dietary starch in the diet may reduce the viability and quality of the oocytes and embryos in dairy cows, thus contributing to an increased

prevalence of embryo mortality rates and a lower pregnancy rate (Leroy *et al*., 2008b). In contrast, high fat based diets in dairy cows were reported to increase the viability and quality of oocytes and also enhance progesterone (P_4) secretion, thus supporting the embryonic developmental competence (Gray *et al*., 2001; Wang *et al*., 2007). Furthermore, manipulating such glucogenic and lipogenic nutrients in diets have been reported to have positive effects on conception and pregnancy maintenance in dairy cows (Lucy, 2007; Garnsworthy *et al*., 2008a, b). However, bovine studies investigating the effect of energy sources and levels on the fertility outcomes from calving to mid- or late lactation are lacking. Also, no studies have been conducted in South Africa to investigate the effect of energy nutrients on the reproductive performances of dairy cows. The assumption of this study was that improving the metabolizable energy intake (high *vs* low) in dairy cows can positively affect fertility outcomes. Secondly, it was hypothesed that manipulating energy sources (starch/fat combination *vs* starch) can improve further conception rate of dairy cows, as insulinogenic diet was reported *in vitro* to yield inferior oocytes and decrease the embryo survival after fertilisation (Armstrong *et al*., 2001; Leroy *et al*., 2008b, c). Therefore, the aim of this study was to evaluate the effect of supplements differing in energy (low *vs* high) and sources (starch *vs* fat) from calving until 154 days postpartum on fertility of Holstein cows in a pasture based system.

6.2. Materials and Methods

6.2.1. Experimental location

The experiment was conducted at the Elsenburg Research Farm, as described in Chapter 3 (3.2.1).

6.2.2. Experimental husbandry, animals and diets

Fresh drinking water was freely available at all times in the trial. The same animals (69 primiparous and 153 multiparous Holstein cows) and experimental diets as described in Chapter 3 (3.2.2) were used. Briefly, any health disruption was monitored during the first 10 days after calving and treated according to the required standard operating procedures either by the herd manager after milking sessions or the veterinarian during the weekly visits to the farm, ensuring a return to a normal reproductive health by 30 DIM. Cows were allocated to three isonitrogenous nutritional programmes according to parity, calving date, LW at calving and milk yield of the previous lactation. Cows were allowed to graze cultivated irrigated kikuyu-ryegrass pastures as a herd and followed a rotation programme to ensure an *ad libitum* DMI. After each milking session (i.e. at 05:30 and 15:00), cows received half of the daily allowance of concentrate supplements, differing in energy levels (low *vs* high) and sources (starch *vs* fat). The control group was offered 7 kg/day of a control concentrate supplement for both primiparous and multiparous cows, while concentrate

supplements in treatment groups were fed at 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively. The control was the standard pasture feeding system and was fed from calving until 154 DIM. The control diet contained low level of energy content (2.47 Mcal ME/kg DM) and provided a total intake of 457, 104 and 40 g/kg of NDF, starch and fat on DM basis, respectively. The supplement in HSLF treatment was a glucogenic concentrate containing maize as the energy source and it was offered from calving to 154 DIM. The HSLF diet contained high level of energy content (2.71 Mcal ME/kg DM) and was fed to provide a total intake of 341, 242 and 35 g/kg of NDF, starch and fat on DM basis, respectively. The HSLF treatment was formulated to increase the circulating glucose and insulin concentrations in order to enhance the postpartum EB status, initiate an early return of oestrous activity, and improve conception rate in cows. The supplements in the HSLF-LSHF treatment were combinations of a glucogenic concentrate offered for the first 60 DIM per treatment HSLF, followed from 61 to 154 DIM by a lipogenic (LSHF) concentrate using wheat bran and Ca-salts of long-chain fatty acids (Megalac rumen bypass fat, Volac International Ltd., UK) as the energy sources. In the HSLF-LSHF combination treatment, the high starch-based diet initially aimed to achieve the same objective of the HSLF treatment during the first 60 DIM. The LSHF diet contained high level of energy content (2.70 Mcal ME/kg DM) and was formulated to provide a total intake of 388, 137 and 58 g/kg of NDF, starch and fat on DM basis, respectively. Secondly, the LSHF diet was fed from 61 DIM to decrease plasma insulin and improve plasma cholesterol in order to increase the ovulatory follicular size, promote embryo development, and improve the conception rate in cows.

6.2.3. Experimental sampling and data collection

The seasons were summer from 15th October to 14th April, with December to February being the hottest months, and winter from 15th April to 14th October, with June to August being the coldest months. Live weights were automatically recorded daily after milking sessions using a calibrated electronic scale. From day 30 postpartum, Kamar detectors (Kamar® Heatmount Detectors, Kamar Products Inc., Zionsville, USA) were attached to the tail-head area of cows. The Kamar detector was positive when cows were showing oestrous behaviours (i.e. mucous vaginal discharge, mounting, sniffing, etc.). Following the heat detection, artificial insemination (AI) was performed after milking sessions according to the am-pm rule, i.e. cows observed on heat before milking were inseminated after the milking session. The AI was done by a single trained inseminator. Kamar detectors were removed at the AI and replaced when a following pregnancy diagnosis was negative. Pregnancy diagnoses were performed by rectal palpation by the veterinarian 35-40 days after the

AI. Postpartum LW traits related to fertility like LW at first service and at conception were derived from LW curves. Reproductive traits of dairy cows were derived from calving and AI dates following pregnancy diagnosis results and measured according to standard fertility practices.

6.2.4. Statistical analysis

Data were grouped according to parity (i.e. primiparous and multiparous cows) and analysed using the year (*Y*), season of calving (*S*), treatment (*T*) as well as the interaction between season of calving and treatment (*ST*) as fixed effects in the statistical model (SAS, 2012). The effects of year and season of calving were included in the statistical model because of the duration of the experiment. The statistical model was as follows:

$Model = \mu + Y_i + S_j + T_k + (ST)_{jk} + \varepsilon_{ijk}$, where

μ = overall mean;

Y_i = the fixed effect of the i^{th} year of calving (i.e. 2012, 2013 and 2014)

S_j = the fixed effect of the j^{th} season of calving (i.e. winter and summer);

T_k = the fixed effect of the k^{th} treatment (i.e. Control, HSLF and HSLF-LSHF);

ST_{jk} = the interaction between levels j^{th} season of calving and k^{th} treatment;

ε_{ijk} = the random experimental error.

Differences of means and standard error (SE) of means between treatments were obtained using the pair wise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

6.3. Results and Discussion

The results of fertility traits and LW changes related to fertility of primiparous and multiparous cows are presented in Table 6.1. No fertility traits in both primiparous and multiparous cows were significantly affected by the effect of year of calving (therefore, *P*-values are not reported in Table 6.1). In addition, the season of calving and the interaction between season of calving and treatment in this study did not affect ($P > 0.05$) fertility traits in both parity groups (Table 6.1). However, De Rensis and Scaramuzzi (2003) reported a decrease in the fertility of postpartum dairy cows inseminated in the summer, compared to cows inseminated during the winter season. Pennington *et al*. (1985) also found that heat stress in dairy cows reduced the duration and intensity of oestrus. The decrease in oestrous activity also led to a decrease in the number of mounts in hot weather compared to the cold weather period, hence resulting in poor detection of oestrus in dairy cows. Kadzere *et al*. (2002) noted that fertility functions are disrupted as dairy cows are less likely to

acclimatize when heat stress conditions are sudden and prolonged in summer. These thermal disruptions alter the hormonal balances, ovarian function, oestrous expression, oocyte health, embryonic development and pregnancy maintenance. Despite the negative effects of seasonal thermal stress observed in other studies, the lack of effects of season of calving and the interaction between season of calving and treatment on fertility traits were possibly related to the low numbers of animals used in this study, masking these effects.

Table 6.1 The effect of nutritional treatments differing after calving in energy levels and sources on LW changes and fertility traits (mean ± SE) recorded for primiparous and multiparous dairy cows during a 22 week postpartum period.

	Concentrates			*P*-values		
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[3]**	***S*[3]**	***ST*[3]**
Primiparous cows						
Number of cows	30	20	19	-	-	-
LW[2] in the 1st week of calving (kg)	511 ± 9	493 ± 11	491 ± 11	0.79	83	NS
LW[2] at first service (kg) (LW[2] change in kg)	488 ± 10 (-23)	497 ± 10 (+4)	504 ± 10 (+13)	0.72	0.36	NS
LW[2] at conception (kg) (LW[2] change in kg)	501 ± 9 (-10)	516 ± 9 (+23)	521 ± 10 (+30)	0.28	0.32	NS
Interval from calving to first service (days)	90 ± 6	81 ± 5	83 ± 7	0.15	0.47	NS
Proportion of cows serviced for the 1st AI[2] at 80 days	0.37 ± 0.12	0.47 ± 0.12	0.56 ± 0.12	0.65	0.19	NS
Proportion of cows pregnant at first service	0.28 ± 0.11	0.37 ± 0.12	0.38 ± 0.13	0.58	0.82	NS
Service/conception	2.55 ± 0.33	2.38 ± 0.36	2.31 ± 0.37	0.63	0.81	NS
Interval days open (days)	139 ± 14	137 ± 16	127 ± 16	0.85	0.35	NS
Proportion of cows pregnant at 100 days	0.22 ± 0.08	0.38 ± 0.11	0.35 ± 0.13	0.21	0.18	NS
Proportion of cows pregnant at 150 days	0.52[a] ± 0.12	0.84[b] ± 0.09	0.81[b] ± 0.10	0.03	0.57	NS
Multiparous cows						
Number of cows	77	38	38	-	-	-
Lactation number	3.82 ± 0.17	3.49 ± 0.22	3.54 ±0.22	-	-	-
LW[2] in the 1st week of calving (kg)	621 ± 7	630 ± 7	620 ± 7	0.53	0.99	NS
LW[2] at first service (kg) (LW[2] change[4] in kg)	599 ± 6 (-23)	621 ± 8 (-9)	611 ± 11 (-9)	0.24	0.26	NS
LW[2] at conception (kg) (LW[2] change[4] in kg)	606 ± 9 (-15)	631 ± 12 (+1)	617 ± 15 (-3)	0.26	0.46	NS
Interval from calving to first service (days)	104 ± 6	98 ± 5	101 ± 6	0.98	0.14	NS
Proportion of cows serviced for the 1st AI[2] at 80 days	0.25 ± 0.05	0.32 ± 0.08	0.38 ± 0.08	0.38	0.25	NS
Proportion of cows pregnant at first service	0.39 ± 0.06	0.44 ± 0.09	0.48 ± 0.08	0.74	0.35	NS
Service/conception	2.13 ± 0.16	2.22 ± 0.27	2.00 ± 0.22	0.83	0.52	NS
Interval days open (days)	140 ± 7	139 ± 10	128 ± 13	0.61	0.17	NS
Proportion of cows pregnant at 100 days	0.26 ± 0.05	0.29 ± 0.08	0.33 ± 0.08	0.57	0.21	NS
Proportion of cows pregnant at 150 days	0.56[a] ± 0.06	0.76[b] ± 0.07	0.81[b] ± 0.12	0.04	0.84	NS

[1]HSLF: High starch-low fat, LSHF: Low starch-high fat.

[2]LW: Live weight, AI: Artificial insemination

[3]*S*: Season, *T*: Treatment, *ST*: Interaction ST.

[4]Change in LW at first service or conception was measured as LW at calving – LW at first service or conception

[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$.

Results showed that nutritional treatments in both primiparous and multiparous groups had no significant effect on some fertility traits, e.g. interval from calving to first service, proportion of cows serviced for the first time within the first 80 DIM, pregnancy rate at first service, service per conception, interval days open and the pregnancy rate at 100 DIM. The present results concur with results on the fertility of lactating dairy cows which were maintained under isocaloric treatments, differing in starch and fat contents (Gilmore *et al.*, 2011). Garnsworthy *et al*. (2009) found that the conception at first AI was improved when feeding on DM basis a high starch-based diet consisting of 182 g/kg of starch until the first increase of plasma P_4, followed by an enriched fat-based diet containing 53 g/kg of fat, compared to others isocaloric treatments. Other studies reported that feeding a high starch-based diet resulted in an earlier resumption of ovulation, earlier oestrous detection and thus advanced timing of the first AI (Fulkerson *et al*., 2001; Gong *et al*., 2002). In this study, the LW of cows at conception did not differ significantly between nutritional treatments in both primiparous and multiparous groups. However, the conception rate at 150 DIM was higher ($P < 0.05$) for cows receiving the HSLF and HSLF-LSHF concentrates in comparison to the control concentrate in both primiparous and multiparous cows. Similarly, Garnsworthy *et al*. (2009), Burke *et al*. (2010) and Reis *et al*. (2012) found that feeding diets enriched in glucogenic and/or lipogenic nutrients improved the pregnancy rate of dairy cows in mid-lactation. In contrast, Gilmore *et al*. (2011) found no improvement in pregnancy rate of dairy cows in mid-lactation when feeding starch and/or fat-based diets, compared to others nutritional treatments.

A successful reproduction programme in dairy cows is a consequence of a chain of events, consisting of the resumption of postpartum oestrous function, the development and ovulation of a healthy oocyte, the conception, the embryonic development, the uterine implantation, the maintenance of pregnancy, and finally the parturition (Garnsworthy *et al*., 2008a; Leroy *et al*., 2008a; Santos *et al*., 2009). A failure at any of these steps results in the failure of a successful conception and embryonic/pregnancy survival (Leroy *et al*., 2008a, b). Because of this, the fertility of dairy cows is considered as a multifactorial trait (Butler, 2003) involving a network of genetic, environmental and managerial factors and their interactions, making it all together difficult to determine the exact reason for a deterioration of fertility (Lucy, 2007; Walsh *et al*., 2011; Bisinotto *et al*., 2012). A decline in the observed expression, intensity and detection of animals in oestrus was reported to negatively affect reproduction outcomes (Van Eerdenburg *et al*., 1996; Kerbrat & Disenhaus, 2004; Lopez *et al*., 2004). Additionally, poor oestrous detection at the farm level also contributes to a decreased fertility rate in dairy cows (Reimers *et al*., 1985). So for instance, a failure in fertility has transpired to increased days from calving until the resumption of oestrus and

days open, decreased conception rates following AI and poor pregnancy survival, as well as to increased incidences of involuntary culling (Gröhn *et al*., 1998; Leroy *et al*., 2008a; Giuliodori *et al*., 2011; Roche *et al*., 2011; Esposito *et al*., 2014). As a result, key causes (i.e. postpartum NEB, postpartum metabolic disorders and diseases, etc.) before or after calving that harmfully impact on fertility have been identified through investigations and discussed in review reports (Jordan & Fourdraine, 1993; Gröhn *et al*., 1995; LeBlanc *et al*., 2002; Lucy *et al*., 2003; Hansen *et al*., 2004; Wathes *et al*., 2007b; Leroy *et al*., 2008a, b, c; Roche *et al*., 2011; Seifi *et al*., 2011; Walsh *et al*., 2011; Bisinotto *et al*., 2012; Evans & Walsh, 2012; Esposito *et al*., 2014; Leroy *et al*., 2014). The effect of nutrition on fertility have demonstrated the importance of dense energy sources like starch and/or fat on enhanced insulin and insulin-like growth factor I (IGF-I) as metabolic signals mediating the critical changes in EB status to optimize reproductive performance of dairy cows (Lammoglia *et al*., 1997; Williams & Stanko, 2000; Garnsworthy *et al*., 2008b, c; Leroy *et al*., 2008c; Santos *et al*., 2009). Beam and Butler (1999) found that an improvement in EB status and a shorter interval to NEB nadir corresponded to fewer days postpartum from calving to ovulation. More specifically, Butler (2000) found that a severe NEB within the first 28 days postpartum was highly correlated with an increased interval from calving to first ovulation. In addition, prolonged periods of NEB were found to be accompanied with suppressions of pulsatile luteinising hormone (LH) and IGF-I levels (Diskin *et al*., 2003), as well as a decrease in oestradiol levels (Garnsworthy *et al*., 2008a). The declines in levels of these hormones suppress the ovarian follicular development (Lucy, 2000) and the expression of oestrus, consequently inhibiting the follicular competence and the ovulation in cows with a NEB (Lucy *et al*., 1991; Butler, 2003). This argument indicates that a longer anoestrous period and/or a failure to conceive and maintain pregnancy occur in dairy cows when the function of their ovaries is compromised by a NEB, through a poor nutritional status (Jorritsma *et al*., 2003; Roche *et al*., 2011).

Increasing levels of starch and/or fat constituents at the expense of forage constituents in diets of dairy cows has been demonstrated as an effective approach for reducing the extent and duration of postpartum NEB (Williams & Stanko, 2000; Garnsworthy *et al*., 2009). Such an approach increases the energy density of the diet (Bargo *et al*., 2003; Schroeder *et al*., 2004; Hills *et al*., 2015) and changes metabolic hormone levels, particularly insulin which stimulates ovarian function in dairy cows (Boland *et al*., 2001; Webb *et al*., 2004; Garnsworthy *et al*., 2008a). Although not measured in this study, feeding high starch-based diets potentially improved the supply of glucogenic precursors (Steinhour & Bauman, 1988) that enhance glucose and insulin levels to subsequently facilitate the uptake of NEFA (Van Knegsel *et al*., 2007a; Garnsworthy *et al*., 2008a, 2009; Lee & Hossner,

2002) and suppress the lipolysis of body fat reserves (Lafontan *et al.*, 2009). High levels of insulin have clearly been shown to stimulate the resumption of the normal endocrine status, inducing an early onset of ovarian activity (Gong *et al*., 2002; Van Knegsel *et al*., 2005). Consistent with these reports, other studies also found that increasing the amount of dietary starch reduced the interval from parturition to first ovulation and therefore initiated earlier postpartum cyclicity (Lucy *et al*., 1991; Beam & Butler, 1997; Burke *et al*., 2010). Butler and Smith (1989) reported that an early onset of ovarian activity allows dairy cows to complete one or more oestrous cycles during the voluntary waiting period before they are due to be bred. Moreover, postpartum cows that ovulate earlier are associated with greater conception rates (Butler, 2000; Ambrose & Colazo, 2007) as these cows are more likely to have normal oestrous cycle lengths and more pronounced oestrous behaviours during the breeding period (Roche, 2006). In contrast, high plasma insulin levels can have harmful effects on oocyte developmental competence, as shown by the rate of blastocyst production following *in vitro* fertilisation and maturation (Armstrong *et al*., 2001; Leroy *et al*., 2008c). Using *in vitro* systems, the disadvantageous effects of insulinogenic diets on oocyte were also found after fertilisation to harm the blastocyst development rate in lactating dairy cows (Fouladi-Nashta *et al*. 2007) and in heifers (Adamiak *et al*., 2005, 2006). When starch was replaced by fat in isocaloric diets, high fat-based diets decreased the plasma insulin levels (Garnsworthy *et al*., 2008b, c, 2009) and had favourable effects on blastocyst development rate in lactating dairy cows (Fouladi-Nashta *et al*., 2009). Furthermore, feeding diets enriched with fatty acids have shown positive effects on the plasma P_4 levels through increased availability of plasma cholesterol, the main precursor for luteal P_4 production (Grummer & Carroll, 1991; Lopes *et al*., 2009). The P_4 hormone arranges the uterine milieu for conceptus growth and development (Robinson *et al*., 2002), while regulating endometrial secretions and structural changes that are essential for suitable embryonic implantation in the uterus (Gray *et al*., 2001; Wang *et al*., 2007). Secondly, P_4 controls the release of hormones such as prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) that may degenerate the corpus luteum and interrupt pregnancy (Bazer *et al*., 1998). More specifically, this hormone is a prerequisite for proper maternal recognition, the establishment and maintenance of pregnancy (Spencer & Bazer, 2002; Wathes *et al*., 2007b; Reis *et al*., 2012). Previous investigations also reported that dietary fat enhanced the energy status (Hightshoe *et al*., 1991; Sklan *et al*., 1994), improved plasma cholesterol levels (Wehrman *et al*., 1991) and affected plasma insulin levels (Lammoglia *et al*., 1997) to induce early postpartum ovarian activity. However, the effects of energy levels and types in this study showed no improvements in the intervals from calving to first service and the proportions of cows pregnant at first service and at 100 DIM in both parity groups.

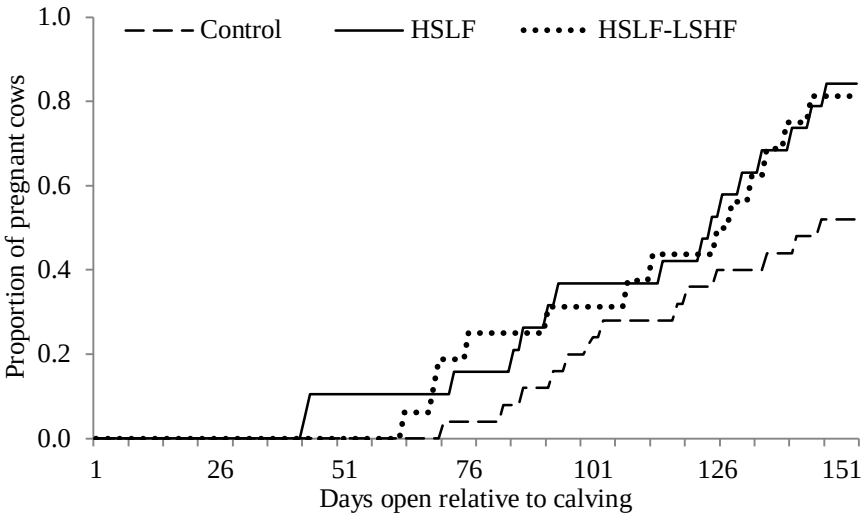

Figure 6.1 The effect of nutritional treatments differing after calving in energy levels and sources on the cumulative proportion of pregnant primiparous cows (mean ± SE) recorded during a 154 day postpartum period.

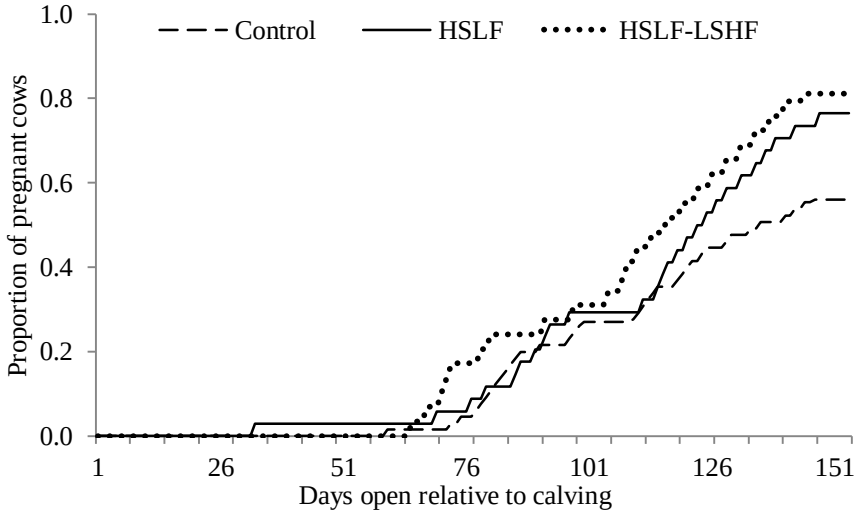

Figure 6.2 The effect of nutritional treatments differing after calving in energy levels and sources on the cumulative proportion of pregnant multiparous cows (mean ± SE) recorded during a 154 day postpartum period.

The proportion of pregnant cows relative to calving between nutritional treatments are presented in Figure 6.1 for primiparous and Figure 6.2 for multiparous groups, respectively. In this study, feeding HSLF and HSLF-LSHF treatments to primiparous and multiparous cows improved ($P < 0.05$) the pregnancy rate at 150 DIM, compared to the control. On the contrary, Gilmore *et al.* (2011) reported that feeding high energy-based diets, containing starch and/or fat, had similar effects on pregnancy rate of mid-lactating dairy cows. McNamara *et al.* (2003) reported also no

difference in the pregnancy rate in mid-lactation, despite significant differences in EB status being observed when supplementing 4 *vs* 8 kg/day of concentrate to postpartum cows on *ad libitum* grass silage. Consistent with the HSLF treatment *vs* the control in this study, Gong *et al*. (2002) found that an insulinogenic diet containing 260 g/kg of starch improved the conception rate compared to the control with 100 g/kg of starch on DM basis. Furthermore, pregnancy rates to first and second services were enhanced when grass silage was supplemented by a concentrate (625 g/kg fresh basis of glucogenic-based ingredients) fed to cows individually, based on milk yields of the previous week, compared to those on a mixed diet containing grass silage and the same concentrate in a 50:50 ratio on a DM basis (Little *et al*., 2016). A pasture-based study that supplemented a larger quantity of starch (38.1 *vs* 17.8% on DM basis) to postpartum dairy cows recorded a reduced occurrence of anovulatory cows, contributing to a greater percentage of the conception rate (94 *vs* 77%) by week six of the breeding season (Burke *et al*., 2010). This enhancement suggested that early or a greater occurrence of oestrus and ovulation before the start of the breeding period has probably benefited the accumulated prevalence of pregnancies in dairy cows (Galvao *et al*., 2010. In this study, the improvement in pregnancy rates in the HSLF treatment can be related to the starch inclusion level of 242 g/kg on a DM basis, supporting the pregnancy success through increased insulin and IGF-I levels, in comparison to 104 g/kg in the control group. Such enhancement suggested that starch level in the HSLF diet was neither marginal nor harmful to affect negatively the conception rate of dairy cows, as reported using *in vitro* systems (Armstrong *et al*., 2001; Leroy *et al*., 2008b, c). In accordance with the HSLF-LSHF treatment *vs* the control in this study, Garnsworthy *et al*. (2009) found that feeding an insulinogenic diet containing 182 g/kg of starch and 39 g/kg on DM basis for the first 50 DIM to encourage cyclicity and followed by a lipogenic diet consisting of 98 g/kg of starch and 53 g/kg of fat on DM basis to promote embryo development, improved the pregnancy rate at 120 DIM in comparison to other isocaloric treatments. However, Gilmore *et al*. (2011) did not find positive results with regard to the conception rate of dairy cows when feeding a similar high-starch/high-fat combination treatment in comparison to other isocaloric diets. Feeding a diet containing 1.1% of fat on DM basis after the expected ovulation reduced pregnancy losses per service in mid-lactating pregnant cows using the transrectal ultrasonography technique at 60 days of pregnancy (Reis *et al*., 2012). Other studies showed that diets enriched with dietary fat reduced pregnancy losses in dairy and beef cattle (Burke *et al*., 1997; Thatcher *et al*., 1997; Mattos *et al*., 2000). The enhancement in pregnancy rates in glucogenic-lipogenic treatment in this study can be attributed to the energy levels of the diets as well as the additive effect of the LSHL diet containing 58 g/kg of fat on DM basis in comparison to 40 g/kg of

fat in the control diet. Besides the maintenance of the energy level, as in the LSHF diet, the addition of fat increased plasma cholesterol levels and P_4 synthesis for pregnancy success and survival (Garnsworthy *et al*., 2008a; 2009; Roche *et al*., 2011). In the pasture-based system used in the current study, HSLF and HSLF-LSHF treatments were designed to provide isocaloric concentrates at inclusion levels of 11.6 and 12.6 kg/day for primiparous and multiparous cows, respectively, compared to 7 kg/day of concentrate for both primiparous and multiparous cows in the control group. The response in pregnancy rates of dairy cows can be attributed in this study to the difference in the energy intake levels provided in HSLF and HSLF-LSHF treatments, improving the nutritional status (i.e. total nutrient intake and ME) as is evident in the postpartum metabolite profiles and LW, compared to the control [as discussed in Chapter 3 (3.3)]. The enhancement in nutritional status of dairy cows in HSLF and HSLF-LSHF treatments had possibly optimised the hypothalamic-pituitary-ovarian-uterus axis and the energy carry-over effect into conception rate in comparison to the control.

6.4. Conclusion

In this study, the pregnancy rates at 150 DIM were significantly improved in grazing primiparous and multiparous cows fed respectively 11.6 and 12.6 kg/day of concentrate in HSLF and HSLF-LSHF treatments, compared to their counterparts being fed a control concentrate at 7 kg/day in the control. Higher inclusion level of concentrates in the HSLF and HSLF-LSHF treatments improved the total nutrient intake and ME in dairy cows, and resulted in an improvement in pregnancy rates in comparison to the control. Further research is required to investigate different sources and inclusion levels of energy nutrients on maternal (i.e. P_4 level, oviductal and uterine environment, placental implantation, etc.) and gestational (i.e. production of interferon tau for pregnancy recognition, embryonic and foetal growth, etc.) factors that improve embryonic/pregnancy survival in dairy cows until the next calving. Also, the effects of other energy sources (i.e. oat, barley, wheat, canola and rapeseed) and their combinations on fertility traits of cows grazing on other irrigated forages (i.e. cynodon, digitaria, paspalum, etc.) produced in Western Cape need to be investigated.

6.5. References

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Chapter 7

The effect of late prepartum feeding programmes on the live weight changes, milk production and fertility traits in the subsequent lactation period of Holstein cows

Abstract

The objective of this study was to evaluate two late prepartum fibre-based diets differing in non-structural carbohydrate (NFC) and protein levels on live weight (LW) changes, milk production and fertility traits of Holstein cows until 120 days postpartum. At 30 days prepartum, pregnant heifers (n=54) and dry cows (n=66) from the Elsenburg herd were allocated to two nutritional treatments. The allocation was done according to parity, expected calving date, LW and milk production during the previous lactation. Prepartum heifers and cows received a similar type and level of a prepartum concentrate supplement, together with an *ad libitum* intake of either unchopped oat hay for the control group or a partial total mixed ration (pTMR) for the treatment group. The pTMR consisted of oat hay (48%), lucerne hay (43%) and soybean oil cake meal (9%). The prepartum treatment diet was formulated to optimise rumen digestion and fermentation, through improved intake of energy and protein per kg on dry matter (DM) basis with the aim of reducing the extent and duration of the periparturient negative energy balance (NEB), compared to the control diet. Following parturition, the control and treatment groups were maintained under similar feeding and managerial conditions. Primiparous and multiparous cows were maintained on *ad libitum* cultivated irrigated kikuyu-ryegrass pastures, supplemented with a postpartum concentrate of 7 kg/day from calving until 120 days in milk (DIM). The prepartum LW of cows was similar between the control and the treatment for the primiparous and multiparous groups. The postpartum LW of the primiparous cows differed ($P < 0.05$) between the control and the treatment, being 488 ± 9 *vs* 507 ± 13 kg, respectively. No difference was observed in terms of postpartum LW of multiparous cows of the control and the treatment groups. The different prepartum diets did not affect milk production and milk composition in the subsequent lactation period. Prepartum diets did not have an effect on fertility parameters of the multiparous cows, whereas primiparous cows receiving the control diet recorded more ($P = 0.04$) days from calving to first service, compared to cows fed the treatment diet during the dry period (DP). Pregnancy rates at 120 DIM for both primiparous and multiparous groups were similar in the subsequent lactation between cows receiving the prepartum oat hay- and pTMR-based diets. Further research is required to investigate different prepartum feeding periods and nutritional

approaches involving different energy levels and sources to encourage postpartum metabolic and hormonal responses that benefit fertility, while sustaining milk production of dairy cows.

7.1. Introduction

During the transition period, dairy cows experience extensive metabolic changes as a result of the increase in energy demands for pregnancy growth or milk production (Bell, 1995). The periparturient period is critically significant to cow welfare and production, as most health problems (e.g. milk fever, retained placenta, lameness, etc.) occur during this time (Drackley, 1999). It is generally accepted that the prepartum nutritional management in dairy cows influences the postpartum metabolic status in early lactation (Damgaard *et al.*, 2013). Towards the end of a DP, dry matter intake (DMI) declines and cows mobilize stored body reserves due to the energy deficiency (Grummer *et al.*, 1995; VandeHaar *et al.*, 1999) in an attempt to meet the postpartum increased demands for body maintenance and production (Rukkwamsuk *et al.*, 1999). The NEB, as evidenced by increased levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) and reduced body condition and LW, is a normal phenomenon occurring in mammals during the periparturient period due to the energy deficiency (Friggens, 2003). However, it is the degree and duration of a NEB, which has been identified as the causal factor of poor lifetime milk production and fertility in dairy cows (Garnsworthy & Webb, 1999; Butler, 2000, 2003; Jorritisma *et al.*, 2003).

The optimal prepartum dietary management strategies regarding the DM and especially the energy intake of prepartum cows relative to their nutritional requirements are still inconclusive (Janovick & Drackley, 2010). Some studies have indicated that feeding an energy dense-based diet to prepartum cows was an encouraging strategy for preparing dairy cows to adapt to the mobilization of body reserves during early lactation (Dann *et al.*, 1999; Rabelo *et al.*, 2005; Andersen *et al.*, 2008; Damgaard *et al.*, 2013). Unrestricted feeding of high energy diets, that are either starch- or fat-based, enhanced DMI of prepartum cows, allowing them to over consume energy relative to their nutritional demands, compared to cows that were fed a lower energy diet (Janovick & Drackley, 2010). However, this overconsumption of energy was reported to be harmful to rumen health and hepatic function in cows (Grum *et al.*, 1996; Rukkwamsuk *et al.*, 1999; Beever, 2006), resulting in a greater decline in DMI during early lactation (Minor *et al.*, 1998; Olsson *et al.*, 1998). Against this background, cows may potentially encounter difficulty or fail to adapt to the postpartum NEB due to metabolic dysfunction (Janovick *et al.*, 2011; Mann *et al.*, 2015) and altered rumen fermentation (Beever, 2006). Furthermore, a severe NEB before calving has been associated during the

subsequent lactation with increased health problems (Duffield *et al*., 2009; Ospina *et al*., 2010a; McArt *et al*., 2012), reduced reproductive success and decreased milk production (Duffield *et al*., 2009; Ospina *et al*., 2010b, c). Other studies investigated the potential benefits of feeding fibre-based diets (> 400g/kg of NDF on DM basis) containing low digestible energy level during the DP (Jouany, 2006; Janovick *et al*., 2011; Vickers *et al*., 2013; Mann *et al*., 2015). These researchers reported that such prepartum diets adjusted the DMI to optimise rumen digestion and fermentation, decreased the mobilization of body reserves, and prevented the hepatic deposition of total lipid and tri-acyl glycerol (TAG). Furthermore, feeding fibre-based diets containing low digestible energy levels to dry cows has several advantages in dairy herds, as evident in fewer health complications, reduced body condition loss, and enhanced reproductive axis after parturition (Drehmann, 2000; Beever, 2006; Jouany, 2006). However, no studies have been conducted in South Africa to investigate the prepartum effect of energy levels and sources on the milk production and reproductive performances of dairy cows in the subsequent lactation. The assumption of this study was that improving the late prepartum intake of energy and protein per kg on DM basis to enhance the energy balance (EB) status of dairy cows can limit the LW loss and improve fertility, while sustaining milk production, during the following lactation. The objective of the study was thus to evaluate the effect of two late prepartum fibre-based diets differing in levels (low *vs* high) of NFC and protein on LW changes, milk production and fertility traits of Holstein cows until 120 days postpartum.

7.2. Materials and Methods

The ethical clearance for this study was obtained from the Western Cape Department of Agriculture (WCDA, Project AP/BR/D/CM31).

7.2.1. Experimental location

The study was conducted at the Elsenburg Research Farm (WCDA), as described in Chapter 3 (3.2.1).

7.2.2. Experimental husbandry, animals and diets

Fresh drinking water was freely available at all times in the trial. Holstein heifers and cows that were expected to calve from February 2014 to July 2015 were included in the study which started at 30 days before the expected calving dates until 120 days after calving. Pregnant heifers (n=54) and dry cows (n=66) were allocated to two prepartum nutritional groups according to parity, expected calving date, LW and milk production during the previous lactation. This was done so that animals

were exposed to similar environmental conditions during the postpartum observation period of the trial. Heifers were assigned based on their LW and excepted calving dates. The LW of heifers at 30 days prepartum was 538 ± 9 and 544 ± 7 kg for the control and treatment groups, respectively. Primiparous cows were 2.19 ± 0.05 and 2.24 ± 0.07 years of age at calving for the control and treatment groups, respectively. Dry cows were assigned according to parity, expected calving date and milk yields in the previous lactation. Live weights of dry cows at 30 days prepartum were 630 ± 7 and 626 ± 5 kg for the control and treatment groups, respectively. The milk yields during the previous lactation of multiparous cows were 8697 ± 131 and 8650 ± 132 kg for the control and treatment groups, respectively. Multiparous cows were 5.41 ± 0.36 and 5.11 ± 0.28 years of age at calving for the control and treatment groups, respectively.

The chemical compositions of feeds and prepartum diets used in the study are presented in Table 7.1. Representative samples of feeds used in the current study were collected weekly, then bulked monthly and analysed for dry matter (DM), ash, fat, neutral detergent fibre (NDF) and crude protein (CP, Nitrogen, Nx6.25) according to standard procedures as described in Chapter 3 (3.2.2). The NFC (consisting of starches, sugars, organic acids, and other reserve carbohydrates such as fructans) in the prepartum diets were estimated according to NRC (2001) as follows:

NFC in g/kg on DM basis = DM x [100 - (NDF% + CP% + Fat% + Ash%)]/ 100, where

DM was expressed in g/kg;

NDF, CP, fat and ash were all expressed in % on DM basis.

Table 7.1 The chemical composition of feeds and estimated chemical composition of prepartum diets used in the study.

						Prepartum diets[3]			
				Concentrates		30 to 14 days		13 days to calving	
Parameters[1]	Oat hay[2]	pTMR[2]	Pasture[2]	Dry period[2]	Postpartum[2]	Control[4]	Treatment[4]	Control[5]	Treatment[5]
DM	932	917	210	891	894	923	911	915	906
Ash	41	69	103	106	72	55	78	69	84
CP	48	152	184	188	179	78	160	107	167
Fat as EE	16	19	34	36	42	20	23	24	26
NDF	689	500	539	179	200	580	432	472	368
NFC	-	-	-	-	-	247	282	299	322
Ca	3	5	5	29	14	9	10	14	15
P	2	3	5	7	7	3	4	4	5

[1]Determined from laboratory analysis, with pTMR: Partial total mixed ration; DM: Dry matter in g/kg as is; CP: Crude protein in g/kg DM; EE: Ether extract in g/kg DM; NDF: Neutral detergent fibre in g/kg DM; NFC: Non-fibre carbohydrates in g/kg DM; Ca: Calcium in g/kg DM; P: Phosphor in g/kg DM. [2]Feeds used the study. [3]Determined from the chemical composition of feeds at an estimated intake level of 14 kg/cow per day and 12 kg/heifer per day on DM basis, respectively. [4]Chemical composition of prepartum diets from 30 to 14 days prepartum. [5]Chemical composition of prepartum diets from 13 days prepartum to calving.

Pregnant heifers and dry cows received a prepartum concentrate mixture, together with an *ad libitum* intake of either unchopped oat hay for the control group or a partial total mixed ration (pTMR, consisting of 48% oat hay + 43% lucerne hay and 9% soybean oil cake meal) for the treatment group. The DP concentrate mixture, containing maize as the main energy source and anionic salts, was given in the morning (08:00) at 3 kg/day for heifers and cows from 30 to 14 days prepartum and thereafter from 13 days prepartum to calving at 6 kg/day and 5 kg/day for cows and heifers, respectively. Levels of prepartum intake were estimated on DM basis at 14 kg/cow per day and 12 kg/heifer per day, respectively. The prepartum treatment was formulated to improve prepartum intake of energy and protein per kg of DM, in order to optimize the ruminal digestion and fermentation and reduce the extent and the duration of the periparturient NEB, compared to the control (Table 7.1). In Figure 7.1, pasture quality parameters over the duration of the postpartum observation period of the study are presented.

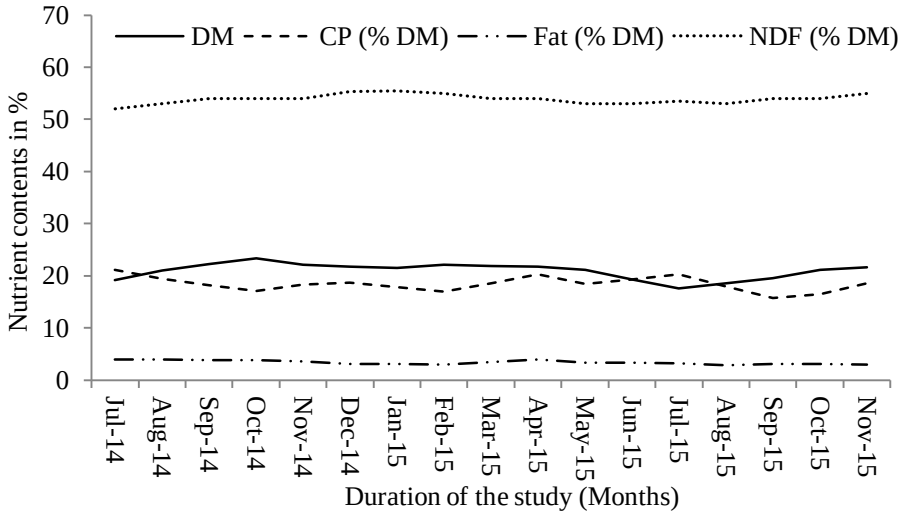

Figure 7.1 A graphical representation of pasture quality parameters monitored during the postpartum observation period of the study. Values are depicted on a DM basis.

Following parturition, any health problem was monitored during the first 10 DIM and treated according to the required standard operating procedures either by the herd manager after milking sessions or the veterinarian during the weekly visits to the farm, to ensure a return to a normal reproductive health by 30 DIM. In addition, both the control and treatment groups were maintained postpartum under similar feeding and management conditions. All animals had *ad libitum* access to cultivated kikuyu-ryegrass pastures being irrigated as required, using a permanent irrigation system. Pastures were grazed in rotation, ensuring unrestricted DMI under typical farming conditions. Both primiparous and multiparous cows were supplemented with a postpartum concentrate of 7 kg/day

from calving until 120 days. After each milking session (i.e. at 05:30 and 15:00), cows received half of the daily allowance of concentrate supplement.

Using the CPM-Dairy software programme (2006), the postpartum diet as presented in Table 7.2 was estimated using an intake level of 22 and 25 kg DM/day for primiparous and multiparous cows, respectively. The diet consisted of 7 kg/day of postpartum concentrate offered in the milking parlour and the rest of the intake being supplied by the pastures, to provide all together 454, 104 and 40 g/kg of NDF, starch and fat on DM basis, respectively.

Table 7.2 Determined composition of the experimental postpartum diet.

Postpartum diet[3]	**Proportion**
Physical	
Ryegrass-Kikuyu pastures (%)	75.0
Postpartum concentrate (%)	25.0
Chemical	
DM[1] (%)	38
ME[2] (Mcal/kg)	2.47
NE_L[2] (Mcal/kg)	1.59
NDF[1] (g/kg DM)	454
NFC[2] (g/kg DM)	255
Starch[2] (g/kg DM)	104
Fat as EE Total[1] (g/kg DM)	40
CP[1] (g/kg DM)	183
RDP[2] (% CP)	62
Ash[1] (g/kg DM)	96
Ca[1] (g/kg DM)	7.1
P[1] (g/kg DM)	5.4

[1]Determined from chemical composition of feeds (see Table 7.1), with DM: Dry matter, NDF: Neutral detergent fibre, EE: Ether extract, CP: Crude protein, Ca: Calcium and P: Phosphor.

[2]Calculated from feed formulation package (CPM-Dairy, 2006), with ME: Metabolizable energy, NE_L: Net energy lactation, NFC: Non-fibre carbohydrate and RDP: Rumen degradable protein.

[3]Postpartum diet was estimated on DM basis using an intake level of 22 and 25 kg DM/day for primiparous and multiparous cows, respectively.

7.2.3. Experimental sampling and data collection

During the prepartum period, pregnant heifers and dry cows were weighed once every week until parturition. Calving seasons were summer from 15 October to 14 April, with December to February being the hottest months, and winter from 15 April to 14 October, with June to August being the coldest months. Daily milk yields and LW were automatically recorded after milking sessions using the Afikim system (AfiMilk) and the calibrated electronic scale, respectively. Daily milk yields and LW data were determined on a weekly basis. Milk samples were collected at the evening and following morning's milking sessions every 35 days, preserved with 2-bromo-2-nitropropane-1, 3

diol and analysed for milk fat, milk protein, milk lactose and milk urea nitrogen (MUN) using the infrared spectrophotometry (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). Milk composition data were determined on a basis of 35 day of intervals. Test periods were from 1 to 35, 36 to 70, 71 to 105 and 106 to 120, respectively. The 4% fat corrected milk (FCM) yield was calculated based on the equation of Gaines (1928): 4% FCM, kg/d = (0.4 x milk yield, kg/d) + (15 x fat, kg/d). The nadir point was defined as days in milk in which the lowest postpartum LW value was recorded (Sakaguchi, 2009). The weekly LW loss was calculated as follows:

$LW\ loss_n = (LW_{week1} - LW_n) \times 100(\%) / LW_{week1}$

Where $LW\ loss_n$ and LW_n were respectively LW loss and LW at *n* weeks after parturition

LW_{week1} was LW within one week after parturition.

The rate of LW loss from calving to LW_{nadir} was estimated in kg/day as follows:

Rate of LW loss from calving to LW_{nadir} = ($LW_{at\ calving}$ - LW_{nadir}) / Number of days to reach LW_{nadir}.

The rate of LW gain from LW_{nadir} to 120 DIM was estimated in kg/day as follows:

Rate of LW gain from LW_{nadir} to 120 DIM = ($LW_{at\ 120\ DIM}$ - LW_{nadir}) / (120 - Number of days to reach LW_{nadir}).

From day 30 postpartum, Kamar heat detectors (Kamar® Heatmount Detectors, Kamar Products Inc., Zionsville, USA) were attached to the tail-head area of cows. After a positive oestrous detection, the artificial insemination (AI) was carried out after either the morning or afternoon milk session by a single trained inseminator. The Kamar detector was positive when cows were showing oestrous behaviours (i.e. mucous vaginal discharge, mounting, sniffing, etc.). Kamar detectors were removed at the insemination and replaced when a following pregnancy diagnosis was negative. Pregnancy diagnoses were performed by rectal palpation by the veterinarian at 35-40 days after the AI. Reproductive traits of dairy cows derived from calving and AI dates following pregnancy diagnostic results and measured according to standard fertility practices.

7.2.4. Statistical analysis

Data were analysed using the PROC MIXED of SAS enterprise guide (SAS, 2012), grouped according to parity, i.e. primiparous or multiparous cows. The statistical model included year (*Y*) effect, calving season (*S*) effect, treatment (*T*) effect, week (*W*) effect of observations and the interaction effects between treatment and week of observation (*WT*) as well as treatment and calving season (*ST*) as fixed effects. The animal effect within treatments was specified as a random effect. The effects of year and season of calving were inserted in the statistical model because of the

duration of the experiment. The measured variables obtained every day within a particular week during the trial were considered as repeated measurements of that particular week block [chapter 3 (3.2.4) for justification]. The statistical model was as follows:

$Model = \mu + Y_i + S_j + T_k + W_l + (ST)_{jk} + (WT)_{kl} + \delta_{(lk)m} + \varepsilon_{ijklm}$, where

μ = overall mean;

Y_i = the fixed effect of the i^{th} year of calving (i.e. 2014 and 2015);

S_j = the fixed effect of the j^{th} season of calving (i.e. summer and winter);

T_k = the fixed effect of the k^{th} treatment (i.e. Control and Treatment);

W_l = the fixed effect of the l^{th} week of observation (i.e.1 to 17)

$(ST)_{jk}$ = the interaction between levels of the j^{th} season of calving and k^{th} treatment;

$(WT)_{kl}$ = the interaction between levels of the l^{th} week of sampling and k^{th} treatment;

$\delta_{(kl)m}$ = the variable effect of the m^{th} block effect in the k^{th} treatment (Repeated statement);

ε_{ijklm} = the random experimental error.

To avoid problems of fitting a covariance structure in the statistical model, prepartum and postpartum LW data were analysed separately. All effects were used to analyse milk yields and the postpartum LW whereas prepartum LW and milk composition traits (i.e. milk fat, milk protein, milk lactose and MUN) were analysed without the repeated statement ($\delta_{(lk)m}$) in the statistical model. The LW traits generated from LW curves, milk yields at peak of lactation as well as the number of days at peak of lactation were analysed using year, calving season, treatment and the interaction between treatment and calving season as only fixed effects in the statistical model. Fertility traits were analysed using year of calving, season of calving and treatment as well as the interaction between treatment and season of calving as fixed effects in the statistical model. Statistical assumptions were described as fixed effects and their interactions were equal to zero with $\delta_{(kl)m} \sim N(0, \sigma_e^2)$ varying independently of ε_{ijklm}. Differences of means and standard error (SE) of means between treatments were obtained using the pair wise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

7.3. Results and Discussion

The results of LW traits of primiparous and multiparous cows are presented in Table 7.3. The effects of year of calving, season of calving and the interaction between the nutritional treatment and the week of observation on prepartum and postpartum LW as well as on postpartum LW loss were not significant for both primiparous and multiparous groups (therefore, *P*-values of the effect

of year of calving and interaction between the nutritional treatments and the week of observation are not indicated in Table 7.3).

Table 7.3 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on live weight traits (mean ± SE) recorded for primiparous and multiparous dairy cows from 8 week prepartum to 17 week postpartum period.

			P-values		
Parameters	**Control**	**Treatment**	T^2	S^2	ST^2
Primiparous cows					
Number of cows	28	26			
LW from week 8 to 1 prepartum (kg)	563 ± 9	570 ± 9	0.49	0.61	NS
LW^1 week 1 after calving (kg)	525 ± 9	540 ± 13	0.08	0.30	NS
LW^1_{nadir} (kg)	452^a ± 8	472^b ± 13	0.04	0.57	NS
LW^2 loss at nadir (kg)	68^a ± 5	85^b ± 8	0.02	0.32	NS
Number of days to reach LW^1_{nadir} (days)	35 ± 3	38 ± 5	0.44	0.22	NS
Rate of LW^1 loss from calving to LW^1_{nadir} (kg/day)	2.07 ± 0.17	2.62 ± 0.27	0.06	0.93	NS
LW^1 at peak MY (kg)	493 ± 10	517 ± 14	0.07	0.48	NS
Rate of LW^1 gain from LW^1_{nadir} to 120 DIM (kg/day)	0.47 ± 0.05	0.49 ± 0.08	0.96	0.24	NS
LW^1 from week 1 to 17 postpartum (kg)	488 ± 9	507 ± 13	<.0001	0.88	0.003
LW^1 loss from week 2 to 17 postpartum (%)	7.65 ± 1.06	9.26 ± 1.13	<.001	0.39	0.004
Multiparous cows					
Number of cows	29	37			
Lactation number	3.97 ± 0.34	3.57 ± 0.24			
LW from week 8 to 1 prepartum (kg)	650 ± 8	645 ± 6	0.51	0.55	NS
LW^1 week 1 after calving (kg)	600 ± 11	614 ± 8	0.11	0.15	NS
LW^1_{nadir} (kg)	537 ± 8	538 ± 8	0.51	0.52	NS
LW^2 loss at nadir (kg)	74 ± 7	86 ± 5	0.09	0.09	NS
Number of days to reach LW^1_{nadir} (days)	32 ± 2	35 ± 2	0.17	0.20	NS
Rate of LW^1 loss from calving to LW^1_{nadir} (kg/day)	2.38 ± 0.22	2.66 ± 0.16	0.30	0.25	NS
LW^1 at peak MY (kg)	569 ± 10	577 ± 9	0.23	0.17	NS
Rate of LW^1 gain from LW^1_{nadir} to 120 DIM (kg/day)	0.18 ± 0.07	0.22 ± 0.05	0.58	0.16	NS
LW^1 from week 1 to 17 postpartum (kg)	579 ± 10	579 ± 8	0.70	0.28	NS
LW^1 loss from week 2 to 17 postpartum (%)	4.91 ± 0.96	6.13 ± 0.79	0.10	0.31	NS

[1]LW: Live weight. [2]*S*: Season, *T*: Treatment, *ST*: Interaction ST.

[a, b] Row means with different superscripts differ significantly at $P < 0.05$.

Prepartum LW of pregnant heifers and dry cows did not differ between the control and the treatment. The interaction between the treatment and time of observation was not significant during the prepartum period whereas both pregnant heifers and dry cows gained LW as calving time approached (Figures 7.2 and 7.4; week of observation $P < 0.05$ not reported in Table 7.3). The increase in prepartum LW in both parity groups was as expected with the increased foetal growth during the late pregnancy. Similar to the present findings, other studies reported no differences in

LW changes when cows were fed fibre-based diets differing in fat and/or starch levels during the prepartum period (Grum *et al*., 1996; Damgaard *et al*., 2013). Fronk *et al*. (1980) studied the eight week prepartum overconditioning of dairy cows, fed 2.7 kg/day of alfalfa hay *ad libitum* and supplemented either by 1.8 kg/day of grain or 6.4 to 8.2 kg/day of grain depending on body condition ratings. These researchers reported that feeding large amounts of grain to prepartum cows improved the digestible energy intake in comparison to those fed low amounts of grain, but their LW changes did not differ between the two groups. Live weight improved in cows overfed energy prepartum in some studies (VandeHaar *et al*., 1999; Dann *et al*., 2006; Douglas *et al*., 2006; Janovick & Drackley, 2010), but no prepartum diet effects were observed in others (Douglas *et al*., 2004).

The bulk of the accumulation of foetal mass (approximately 60% of a calf LW at birth) normally occurs during the last two months of the pregnancy (Bauman & Currie, 1980). Through homeorhetic controls, the imposition of pregnancy during this period requires the specific nutrients (i.e. glucose and amino acids) that are partitioned not only for the development of the foetus, but also for the growth of the foetal membranes, the gravid uterus, and the mammary gland. In addition, Everitt (1964) reported that a foetus of a ruminant is more vulnerable than that of many other species to maternal undernutrition stresses, impeding the normal foetal growth. Thus, maternal adaptions during the late pregnancy partitioned nutrients in heifers and dry cows required for their own maternal growth and/or replenishment of protein and energy reserves to meet the foetal requirements (Bauman & Currie, 1980). In this study, the lack of prepartum diets differing in NFC and protein levels on the prepartum LW of heifers and dry cows can be related to the prioritization of nutrients and metabolizable energy (ME) to support the pregnancy requirements.

Changes in LW and LW loss over time are illustrated in Figures 7.2 and 7.3 for primiparous cows and Figures 7.3 and 7.4 for multiparous cows, respectively.

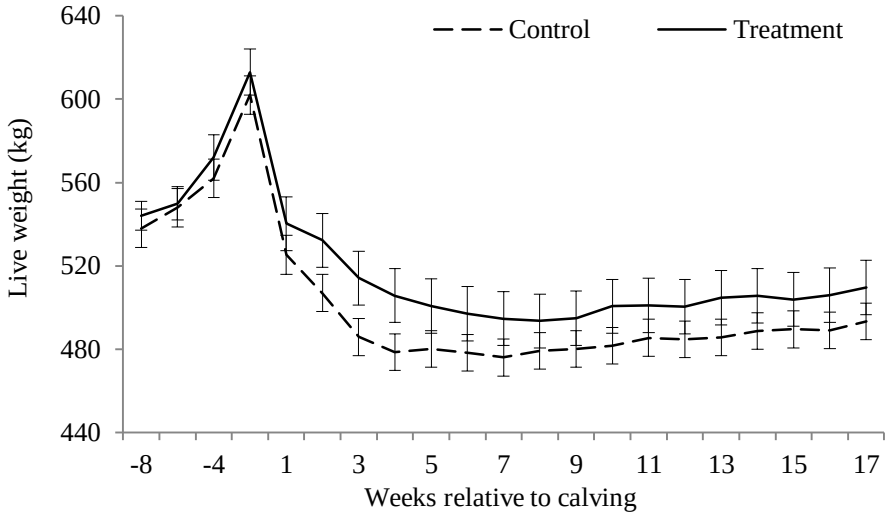

Figure 7.2 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on live weight (mean ± SE) recorded for primiparous dairy cows during an eight week prepartum to 17 week postpartum period.

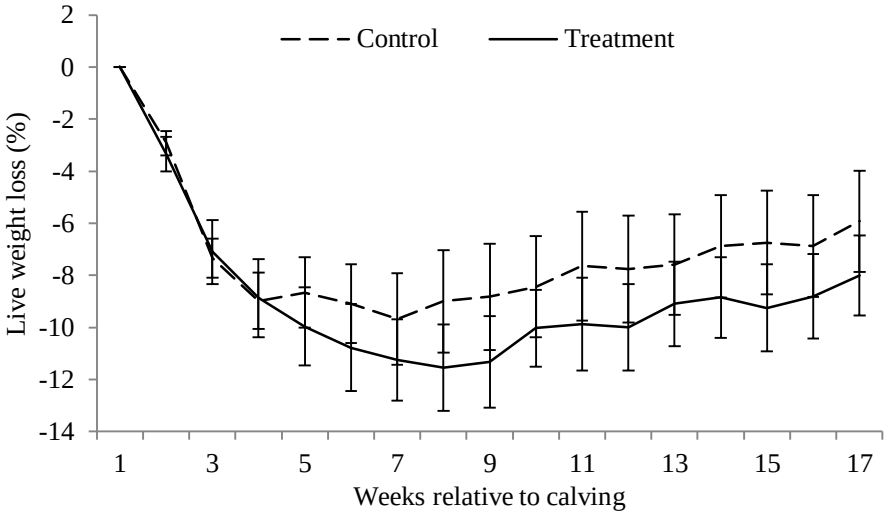

Figure 7.3 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on postpartum live weight loss (mean ± SE) recorded for primiparous dairy cows during a 17 week postpartum period.

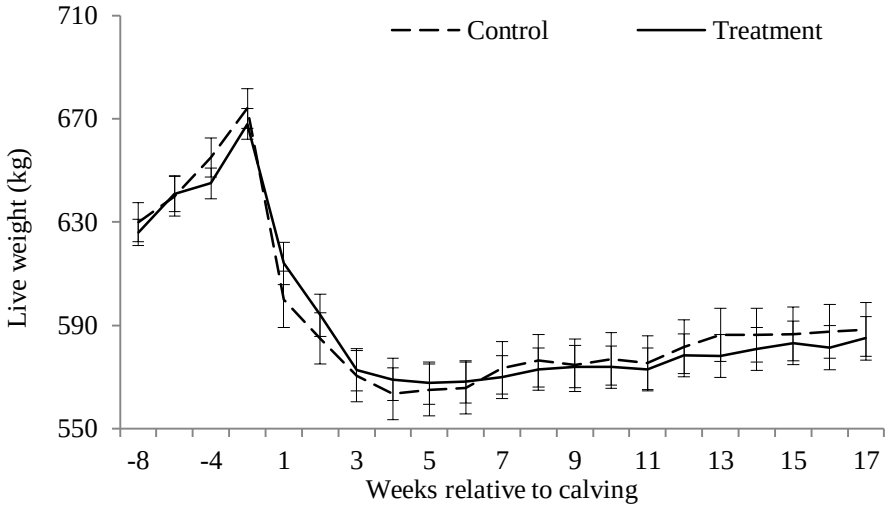

Figure 7.4 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on live weight (mean ± SE) recorded for multiparous dairy cows during an eight week prepartum to 17 week postpartum period.

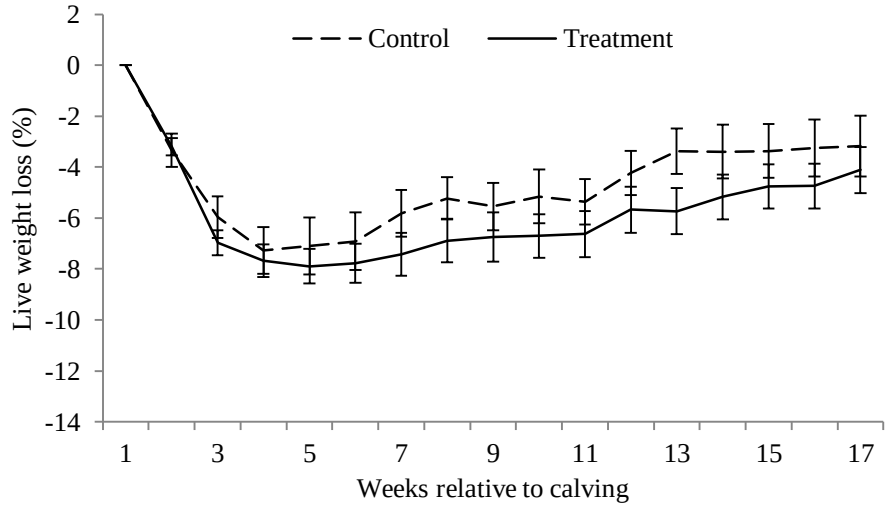

Figure 7.5 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on postpartum live weight loss (mean ± SE) recorded for multiparous dairy cows during a 17 week postpartum period.

The prepartum nutritional effects were not detected on postpartum LW and LW loss for multiparous cows (Figures 7.4 and 7.5). However, the postpartum LW improved ($P < 0.05$) in the treatment in comparison to the control group in primiparous cows (Figure 7.2), being 507 ± 13 *vs* 488 ± 9 kg, respectively. The LW loss in primiparous cows was higher ($P < 0.05$) in the treatment in comparison to the control (Figure 7.3). Furthermore, the LW $loss_{nadir}$ in primiparous cows decreased ($P < 0.05$) in the control compared to the treatment but cows weighed at nadir 20 kg more ($P < 0.05$) in the treatment in comparison to the control group. In contrast to the current finding, previous studies where cattle were fed different energy levels and sources in prepartum diets and a similar

postpartum diet showed no differences in postpartum LW changes (Grum *et al*., 1996; Agenäs *et al*., 2003; Douglas *et al*., 2004, 2006; Guo *et al*., 2007; Andersen *et al*., 2008; Ballou *et al*., 2009; Damgaard *et al*., 2013). In the current study, the lack of effect of prepartum nutritional treatments on the postpartum LW can be attributed to the increased ability of mature multiparous cows (parity > 3) to adapt to the postpartum energy stress, compared to young and still growing primiparous cows.

Table 7.4 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on milk production and milk composition (mean ± SE) recorded for primiparous and multiparous dairy cows during a 17 week postpartum period.

			P-values				
Parameters	**Control**	**Treatment**	T^2	S^2	W^2	TW^2	ST^2
Primiparous cows							
Number of cows	28	26					
Milk yield (MY[1], kg/day)	20.3 ± 0.15	20.3 ± 0.22	0.80	<.001	<.001	NS	NS
4% FCM[1] yield (kg/day)	20.8 ± 0.62	20.8 ± 0.91	0.89	<.001	<.001	NS	NS
MY[1] at peak of lactation (kg/day)	27.6 ± 0.84	28.1 ± 0.82	0.65	0.18	-	-	NS
Number of days to reach peak MY[2] (days)	26 ± 3	30± 3	0.26	0.16	-	-	NS
Fat (%)	3.89 ± 0.07	3.92 ± 0.10	0.49	0.01	<.001	NS	NS
Protein (%)	3.04 ± 0.04	3.02 ± 0.05	0.87	0.63	<.001	NS	NS
Lactose (%)	4.80 ± 0.03	4.75 ± 0.04	0.29	0.11	0.09	NS	NS
Fat: Protein ratio	1.28 ± 0.02	1.30 ± 0.03	0.41	0.25	0.31	NS	NS
Milk Urea Nitrogen (MUN, mg/kg)	13.0 ± 0.51	12.7 ± 0.69	0.12	0.08	0.11	NS	NS
Multiparous cows							
Number of cows	29	37					
Lactation number	3.97 ± 0.34	3.57 ± 0.24					
Milk yield (MY[1], kg/day)	29.2 ± 0.24	28.5 ± 0.18	0.11	<.001	<.0001	NS	NS
4% FCM[1] yield (kg/day)	29.7 ± 0.88	29.0 ± 0.71	0.22	<.001	<.0001	NS	NS
MY[1] at peak of lactation (kg/day)	39.7 ± 0.98	39.4 ± 0.87	0.83	0.20	-	-	NS
Number of days to reach peak MY[2] (days)	24 ± 3	27 ± 2	0.25	0.98	-	-	NS
Fat (%)	3.68 ± 0.05	3.80 ± 0.05	0.08	0.38	0.0007	NS	NS
Protein (%)	2.94 ± 0.03	2.94 ± 0.03	0.9	0.77	<.0001	NS	NS
Lactose (%)	4.70 ± 0.02	4.74 ± 0.02	0.53	<.001	0.53	NS	NS
Fat: Protein ratio	1.25 ± 0.02	1.29 ± 0.02	0.11	0.46	0.6	NS	NS
Milk Urea Nitrogen (MUN, mg/kg)	12.4 ± 0.45	12.3 ± 0.44	0.93	0.64	0.001	NS	NS

[1]MY: Milk yield, FCM: fat corrected milk.

[2]*S*: Season of calving, *W*: week of observation, *T*: Treatment, *ST*: Interaction ST, *TW*: Interaction TW.

[a, b] Row means with different superscripts differ significantly at $P < 0.05$.

The results of the milk production of primiparous and multiparous cows in the subsequent lactation are presented in Table 7.4. The effect of year of calving was not significant on milk production in both primiparous and multiparous cows (therefore, *P*-values are not reported in Table 7.4). A

calving season effect was recorded ($P < 0.05$) on milk production, with high yields being produced in winter compared to the summer season in both the primiparous and multiparous cows. In the primiparous groups, milk yields between the control and the treatment were 21.4 ± 0.22 and 21.05± 0.37 kg/day, respectively, in winter compared to 19.2 ± 0.20 and 19.0 ± 0.29 kg/day in the season summer. In the multiparous groups, milk yields for the control and the treatment were 32.2 ± 0.42 and 31.8± 0.27 kg/day, respectively, in winter compared to 26.2 ± 0.25 and 25.2 ± 0.23 kg/day in the summer season. This was in agreement with the findings of Mostert *et al.* (2001) reporting highest daily milk yield in dairy cows in the cooler months in South Africa. Similarly, Barash *et al.* (1996) found that cows calving in winter produce more milk per lactation than those calving in summer in Mediterranean regions. Kadzere *et al.* (2002) reported that heat stress in the summer season disrupts the hypothalamus to trigger the satiety centre which inhibits the appetite centre, resulting in a decrease in DMI and rumination, and later in milk production.

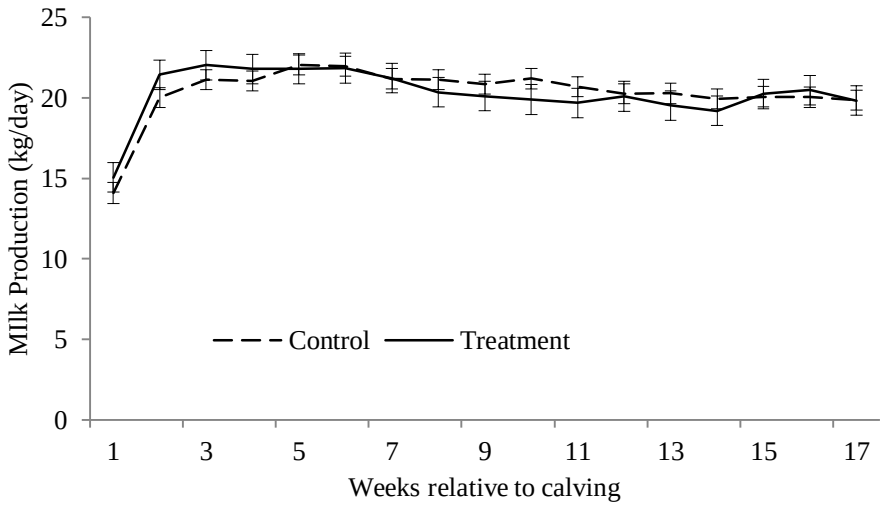

Figure 7.6 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on the milk yield (mean ± SE) recorded for primiparous dairy cows during a 17 week postpartum period.

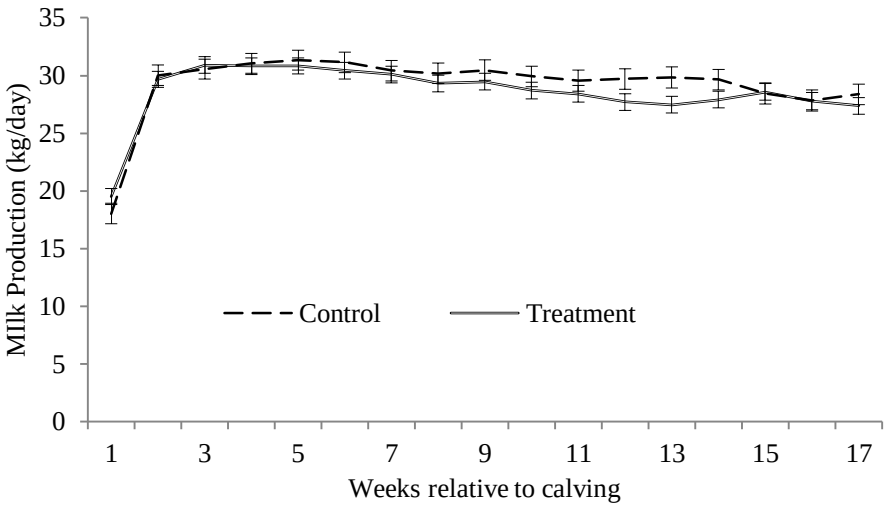

Figure 7.7 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on the milk yield (mean ± SE) recorded for multiparous dairy cows during a 17 week postpartum period.

The effect of prepartum nutritional treatments on milk yield and 4% FCM yield was similar between the treatment and the control in both primiparous and multiparous cows (Table 7.4). Figures 7.6 and 7.7 showed the milk production response over time in primiparous and multiparous cows, respectively. Although milk yield increased over time ($P < 0.05$), no interaction between treatment and week of observation was recorded for both the primiparous and multiparous cows. No differences in milk yield at peak of lactation between the control and the treatment were detected for the primiparous and multiparous cows. In addition, the number of days to reach peak of lactation were similar for the control and treatment groups in both parity groups. Similarly to these findings, no effects on milk yield were detected when corn silage was partially substituted at 21.3% with corn grain in the prepartum diets of dairy cows (Mashek & Beede, 2000). In addition, prepartum diets made of low (5.2 kg of whole-plant wheat silage on DM basis/day) *vs* high (3.6 kg of wheat bran + 0.012 kg of urea + 4.7 kg of corn silage, on DM basis/day) supplements for cows grazing natural pastures had no effect on milk yields in the subsequent lactation (Cavestany *et al*., 2009b). Also, milk yield was unchanged in the subsequent lactation when feeding prepartum fibre-based diets containing high fat or grains (Grum *et al*., 1996). Similarly, Damgaard *et al.* (2013) found no improvement in milk yield when fibre-based diets containing 2.7% of fat and 12.8% of starch in the control and 6.4% of fat and 10.8% of starch in the treatment were fed on DM basis for 56 days prepartum. Fronk *et al*. (1980) studied the postpartum performances of dairy cows maintained in the eight week prepartum on 2.7 kg/day of alfalfa hay *ad libitum* and supplemented either by 1.8 kg/day of grain or 6.4 to 8.2 kg/day of grain depending on body condition ratings. These researchers

reported that prepartum diets did not improved milk yield of dairy cows in the subsequent lactation. In contrast, Cavestany *et al.* (2009a) found that supplementing 3.5 kg/day of cracked corn grain to dry cows on hay mixtures (12.4% CP and 60.1% NDF) fed *ab libitum* increased milk production in the subsequent lactation, compared to unsupplemented cows.

In this study, milk composition traits were influenced by the calving season (Table 7.4), with the milk fat content in primiparous cows and milk lactose content in multiparous cows being higher in winter compared to summer, respectively. Milk fat contents in primiparous group were 4.11 ± 0.11% in winter and 3.77 ± 0.8% in summer ($P = 0.01$), while milk lactose contents of multiparous cows were 4.79 ± 0.02% in winter and 4.66 ± 0.02% in summer ($P < .001$), respectively. This was in agreement with studies reporting changes in milk components of dairy cows as result of a change in calving season (Mostert *et al*., 2001; Kadzere *et al*., 2002). However, milk composition traits (i.e. milk fat, milk protein, milk lactose and MUN) were not affected by the prepartum diet in both the primiparous and multiparous cows (Table 7.4). Fronk *et al*. (1980) found no effect on milk composition when prepartum dairy cows were fed 2.7 kg/day of alfalfa hay *ad libitum* and supplemented either by 1.8 kg/day of grain or 6.4 to 8.2 kg/day of grain depending on body condition ratings. Similarly, no effects on milk composition were recorded when corn silage was partially substituted at 21.3% with corn grain in the TMR diet of prepartum dairy cows (Mashek & Beede, 2000). In addition, no effect of prepartum fat-based diets was also detected on milk fat content (Douglas *et al*., 2004; Andersen *et al*., 2008). Cavestany *et al*. (2009b) found that prepartum fibre-based diets consisting of low (5.2 kg of whole-plant wheat silage on DM basis/day) *vs* high (3.6 kg of wheat bran + 0.012 kg of urea + 4.7 kg corn silage, on DM basis/day) level supplements on cows grazing natural pastures had no effect on milk protein content, but resulted in increased milk fat content with high supplementation. Similarly, other studies found no difference in milk protein but decreased milk fat content when fat was added into the prepartum fibre-based diet fed in a TMR system (Grum *et al*., 1996; Damgaard *et al.*, 2013). Whether different energy sources in prepartum diets might have indirectly affected the synthesis of milk contents postpartum in the udder was investigated by Grum *et al.* (1996), but remains to be elucidated further.

The effects of prepartum diets on milk production of the subsequent lactation are conflicting, with some studies reporting a positive effect on milk yields (Ingvartsen & Andersen, 2000; Cavestany *et al.*, 2009a) and milk composition (Cavestany *et al*., 2009b; Grum *et al*., 1996; Damgaard *et al*., 2013) and others showing no effects (Mashek & Beede, 2000; Keady *et al*., 2001; Doepel *et al*., 2002; Agenäs *et al*., 2003; Guo *et al*., 2007; Mann *et al*., 2015). Primarily, it was argued that

prepartum diets affect the cow's body condition at calving, which has been positively related to cow health (Beever, 2006) and early milk production in the subsequent lactation (Grainger *et al.*, 1982). As a result of larger changes into cow's body condition, cows overfed energy prepartum recorded greater levels of plasma NEFA and BHB, a greater hepatic accumulation of total lipid and TAG and an increased incidence of ketosis after calving (Mann *et al.*, 2015). Conversely, cows calving in poor condition mobilize more energy towards restoring body reserves and less towards milk synthesis than cows calving in good condition (Grainger *et al.*, 1982). Low DMI around calving affecting the EB status negatively seems to have influenced milk yield and milk composition of dairy cows in early lactation (Grummer *et al.*, 2004; Drackley *et al.*, 2005). Secondly, another study pointed out that levels and types of prepartum diets had minor effects on postpartum DMI, hence partitioning very little nutrients towards milk production in early lactation (Cavestany *et al.*, 2009b). Finally, others established unclear or no carry-over effects of prepartum diets on the postpartum plasma and hepatic metabolites as well as on milk synthesis in the udder (Damgaard *et al.*, 2013; Mann *et al.*, 2015). These researchers disputed that the limitation in prepartum DMI, which is attributed to the rumen compression caused by the increased foetal growth towards the end of the gestation (Ingvartsen *et al.*, 1992), did not carry-over to the postpartum period. In this study, the lack of differences in milk production of dairy cows can be attributed to the similar postpartum partitioning of ME and milk precursors to the mammary gland for milk synthesis in both parity groups.

The results of postpartum reproductive performances of primiparous and multiparous cows are presented in Table 7.5. The effects of year of calving and season of calving did not affect any fertility outcomes (therefore, *P*-values of the year effect are not reported in Table 7.5). The lack of effects of season of calving was possibly related to the low number of animals used in this study, masking these effects. In contrast to the present finding, De Rensis and Scaramuzzi (2003) noted a decrease in the fertility of postpartum dairy cows inseminated in the summer, compared to those inseminated in winter. Pennington *et al.* (1985) reported that heat stress in dairy cows decrease the duration and intensity of oestrous activity leading to a decrease in the number of mounts in hot weather compared to cold weather, hence poor detection of oestrus. Supporting these arguments, fertility functions were reported to be disrupted as dairy cows are not comfortable during sudden and prolonged heat stress conditions in the Mediterranean summer (Kadzere *et al.*, 2002). These disruptions then affect the hormonal balances, ovarian function, oestrous expression, oocyte health, conception, embryonic development and pregnancy maintenance of dairy cows.

Table 7.5 The effect of prepartum nutritional treatments differing non-fibre carbohydrate and protein levels on LW changes and fertility traits (mean ± SE) recorded for primiparous and multiparous dairy cows during an eight week prepartum to 17 week postpartum period.

			P-values		
Parameters	**Control**	**Treatment**	T^2	S^2	ST^2
Primiparous cows					
Number of cows	28	26			
LW[1] in the 1st week of calving (kg)	525 ± 9	550 ± 12	0.08	0.3	NS
LW[1] at first service (kg) (LW[1] change in kg)	492 ± 7 (-33)	501 ± 11 (-49)	0.45	0.97	NS
LW[2] at conception (kg) (LW[1] change in kg)	501 ± 7 (-24)	498 ± 9 (-52)	0.55	0.28	NS
Interval from calving to first service (days)	117[a] ± 9	86[b] ± 8	0.04	0.69	NS
Proportion of cows serviced for the 1st AI[1] at 80 days	0.32 ± 0.09	0.38 ± 0.09	0.67	0.29	NS
Proportion of cows pregnant at first service	0.25 ± 0.08	0.31 ± 0.09	0.46	0.5	NS
Service/conception	2.34 ± 0.32	2.08 ± 0.34	0.56	0.1	NS
Proportion of cows pregnant at 120 days	0.34 ± 0.06	0.46 ± 0.09	0.35	0.53	NS
Multiparous cows					
Number of cows	29	37			
Lactation number	3.97 ± 0.34	3.57 ± 0.24			
LW[1] in the 1st week of calving (kg)	600 ± 11	614 ± 8	0.11	0.15	NS
LW[1] at first service (kg) (LW[1] change[3] in kg)	588 ± 8 (-12)	577 ± 11 (-37)	0.93	0.63	NS
LW[2] at conception (kg) (LW[1] change[3] in kg)	589 ± 8 (-11)	599 ± 8 (-15)	0.71	0.75	NS
Interval from calving to first service (days)	104 ± 6	99 ± 6	0.57	0.66	NS
Proportion of cows serviced for the 1st AI[1] at 80 days	0.27 ± 0.09	0.35 ± 0.08	0.31	0.97	NS
Proportion of cows pregnant at first service	0.34 ± 0.06	0.29 ± 0.08	0.42	0.92	NS
Service/conception	2.48 ± 0.24	2.39 ± 0.21	0.65	0.15	NS
Proportion of cows pregnant at 120 days	0.42 ± 0.09	0.38 ± 0.08	0.33	0.26	NS

[1]LW: Live weight, AI: Artificial insemination. [2]*S*: Season, *T*: Treatment, *ST*: Interaction ST.

[3]Change in LW at first service or conception was measured as LW at calving – LW at first service or conception.

[a, b] Row means with different superscripts differ significantly at $P < 0.05$.

No prepartum diet effects were recorded between control and treatment groups for the both primiparous and multiparous cows regarding the proportion of cows serviced for the first time within the first 80 DIM, pregnancy rates at first service and cow LW at first service (Table 7.5). In addition, no prepartum diet effects were recorded in the interval from calving to first service of multiparous cows, while cows in the control group had a larger interval from calving to first service (*P < 0.05*) compared to those in the treatment group for primiparous animals (Table 7.5). Butler and Smith (1989) reported that early resumption of oestrus in dairy cows allows them to complete one or more oestrus cycles before breeding. Moreover, an early ovarian cyclicity in postpartum cows was reported to be associated with an improved conception rate (Butler, 2000; Ambrose & Colazo, 2007; Galvao *et al*., 2010), as these cows are more likely to have normal oestrous cycle lengths and more pronounced oestrous behaviours during the breeding period (Roche, 2006). However, the

improvement in the interval from calving to first service in the treatment group did not affect pregnancy rates at 120 DIM of primiparous cows in the current study. Similarly, prepartum diet effects in multiparous cows showed no postpartum differences in the proportion of cows pregnant at 120 DIM. Similar to these results, other investigations found that prepartum diets differing in energy levels and sources did not affect postpartum pregnancy rates of dairy cows in the subsequent lactation (McNamara *et al*., 2003; Burke *et al*., 2010).

Very few investigations studied the effect of prepartum diets on the fertility of dairy cows in the subsequent lactation (Grummer, 2007). Additionally, these trials had inadequate numbers of dairy cows per treatment to have statistical meaning to accurately evaluate the prepartum diet effect on the postpartum reproductive performances (Drackley & Cardoso, 2014). Most of these studies reported the absence of any effect on postpartum fertility traits when prepartum diets were altered to manipulate periparturient energy intakes (Flipot *et al.*, 1988; Whitaker *et al*., 1993; Kruip *et al*., 1998; Keady *et al*., 2001; McNamara *et al*., 2003; Pushpakumara *et al.*, 2003; Burke *et al*., 2010). The number of cows per treatment in these studies varied from 10 to 45. In contrast, increasing dietary fat in prepartum diets from 2.9 to 4.9% with granular long chain saturated fatty acids improved the pregnancy rate at 220 DIM from 58 to 86% (Frajblat & Butler, 2003). Cavestany *et al.* (2009b) found that the proportions of grazing cows that ovulated from the first dominant follicle postpartum were similar when fed prepartum diets consisting of low (5.2 kg of whole-plant wheat silage on DM basis/day) *vs* high (3.6 kg of wheat bran + 0.012 kg of urea + 4.7 kg corn silage, on DM basis/day) levels of concentrate supplements in both parity groups. However, the interval from calving to first ovulation in the same study decreased in cows that received high *vs* low levels of supplements in the multiparous cows. Castro *et al.* (2012) studied the effect of the metabolic and energy status during the DP on the postpartum resumption of ovarian activity in dairy cows, using the messenger RNA abundance of various gluconeogenic enzymes and metabolic hormones in the liver. These researchers found that the postpartum ovarian function in cows was negatively affected by the low prepartum energy status as evident in low levels of glucose, insulin, insulin-like growth factor I (IGF-I) and thyroxine. Furthermore, the prepartum energy status was reflected in postpartum anovulatory cows by timely declines in hepatic messenger RNA abundance of metabolic factors such as I-R, PC and PEPCKm (Insulin receptor, pyruvate carboxylase and phosphoenolpyruvate carboxykinase-mitochondrial, respectively). Using a Cox proportional hazard model on data of 7 experiments from 1993 to 2010, Cardoso *et al.* (2013) investigated the effect of prepartum energy levels (low: limited NE_L intake to $\leq$ 100% of requirement *vs* high: NE_L intake to > 100% of requirement) on reproductive performance in dairy cows in the subsequent lactation.

These researchers found that cows fed a prepartum low energy diet during the close up DP prevented the mobilisation of body reserves and increased NE_L intake during the first four weeks after calving. Such postpartum biological improvements in the same study resulted in reduced hepatic fat deposition and decreased days open in comparison to those on prepartum high energy diet.

Dairy heifers usually calved for the first time around 24 months of age, making them not physically mature at this age (Coffey *et al*., 2006). Pregnant heifers need nutrients for their own growth in addition to that of the developing calf before calving or milk synthesis after calving (Wathes *et al*., 2007; McArt *et al*., 2013), making them more vulnerable to adapt to the periparturient NEB in comparison to multiparous cows. Normally, the NEB commences a few days before parturition, reaches a NEB nadir 2 to 4 weeks postpartum, and the EB becomes positive again by 10 to 12 weeks after calving (Butler, 2003). This energy deficit results in a loss of LW in dairy cows (Jorritsma *et al*., 2003; Van Straten *et al*., 2008). Additionally, a carry-over effect of the NEB status suppresses the luteinizing hormone (LH) surge and decreases ovarian responsiveness to LH stimulation (Beam & Butler, 1999; Butler, 2000), both of which result in delays in the resumption of cyclicity (Butler *et al*., 2006; Giuliodori *et al*., 2011), reduced oestrous expression (Lopez *et al*., 2004) and diminished viability and quality of the ovulating follicle (Lucy *et al*., 1991). A possible explanation for different postpartum intervals from calving to first service in primiparous cows can be related to the ability of the prepartum treatment to improve the nutritional status and the EB status, as was evident in increased postpartum LW in the treatment, compared to the control (Table 7.3 and Figure 7.2). In multiparous cows (parity > 3), the lack of effect of prepartum nutritional treatments on the postpartum interval from calving to first service can be attributed to the increased hepatic adaptation to postpartum energy stress, compared to young and still growing primiparous cows. The lack of differences in pregnancy rates at 120 DIM in both parity groups can be related to the prepartum feeding period used in the current trial, compared to a conventional DP of 56 to 60 days. The prepartum period used in this study had possibly not been sufficient to encourage postpartum metabolic and hormonal responses that improve the conception success in dairy cows in the subsequent lactation. Another possible reason explaining the lack of differences on pregnancy rate may be attributed to the low number of animals per treatment used in the trial.

7.4. Conclusion

In this study, results showed that late prepartum diets had no effect on milk yield and milk composition of Holstein cows during the subsequent lactation. Although primiparous cows on an

oat hay diet had a significantly longer interval from calving to first service compared to those on the pTMR during the late prepartum period, the conception rate at 120 DIM of dairy cows was similar between the treatment and the control in both parity groups. Different prepartum feeding periods and nutritional strategies involving different energy types and levels to stimulate postpartum metabolic and hormonal responses that benefit fertility, while sustaining milk production of dairy cows, warrant further investigation.

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Chapter 8

General conclusions and Recommendations

The decline in fertility of dairy cows influences not only the culling rate, the size of the herd, the cow longevity, but also the financial return of milk production and the sustainability of dairy farming enterprise. Amongst other solutions, nutrition of dairy cattle represents a key in enhancing productivity of dairy cows. Besides ensuring the rumen health, adequate nutrition supports the milk production levels, restores body reserves and live weight (LW) between lactations, and enables dairy cows to calve at regular intervals. However, the costs of nutrition represent almost 70% of the total input costs in dairy farming and hence, maintaining poor or non-fertile and/or lower milk producing cows in the dairy herd is evidently non-economical. Non-fibre carbohydrates and/or fat are important energy sources in the formulation of diets of dairy cattle. Feeding different levels and types of these energy sources during the prepartum and/or postpartum periods create potential means for dairy cows to adjust their dry matter intake (DMI) and nutrient intake, while controlling their energy balance (EB) status and productivity. Some positive effects such as improvements of periparturient metabolic adaptation, welfare, milk production and fertility have been reported in dairy cows. The existing literature, reporting such effects, serves as evidence that energy sources can indeed be manipulated when specific feedstuffs have been included in prepartum and postpartum diets to improve the metabolism and productive responses of dairy cows.

The emphasis of prepartum and postpartum energy nutrition on milk production and fertility of dairy cows formed the basis of this study. The first aim of this study was to investigate the effect of nutritional treatments differing after calving in energy levels (low *vs* high) and sources (starch *vs* fat) on plasma metabolites and LW changes, milk production and milk composition, ovarian follicular dynamics and oestrous activity, and fertility of Holstein cows. The second aim of this study was to evaluate the effect of two late prepartum fibre-based diets differing in levels (low *vs* high) of non-fibre carbohydrate (NFC) and protein on LW changes, milk production and milk composition, and fertility traits of Holstein cows in the subsequent lactation in a pasture-based system. The findings are summarized in the sections below, with future recommendations.

Nutritional treatments differing after calving in energy levels and sources on blood metabolite profiles and live weight changes as an indication of the nutritional status in Holstein cows

Results on plasma metabolites showed all cows mobilised their body reserves before and after calving. Compared to the control, HSLF and HSLF-LSHF treatments significantly decreased

plasma NEFA and BHB levels in primiparous cows and increased plasma urea levels in both primiparous and multiparous cows. In addition, HSLF and HSLF-LSHF treatments significantly decreased postpartum LW loss of primiparous and multiparous cows in comparison to the control. These biological responses indicated an improved nutritional status of dairy cows in HSLF and HSLF-LSHF treatments, probably favouring somatotropic axis and metabolic responses that benefit the restoration of body reserves and LW, in comparison to the control. Continuous monitoring of LW can be exploited at the farm level as a proxy to identify the physiological well-being and the nutritional status of dairy cows in response to managerial purposes (e.g. nutritional treatment according to performance or to prevent undesired LW loss or gain). Further research is needed to investigate the point at which long-term changes in postpartum LW (i.e. LW $loss_{nadir}$ and number of days to reach the LW_{nadir}) act as stressors to upset milk responses, suppress the gonadotropic axis, and reduce fertility outcomes of dairy cows.

Postpartum energy levels and sources on milk production and milk composition of Holstein cows

Findings on productive responses revealed that feeding HSLF and HSLF-LSHF of concentrates primiparous and multiparous Holstein cows significantly increased milk yield in comparison to their counterparts in the control. In the primiparous group, the HSLF diet enhanced milk protein content in comparison to the control and HSLF-HFLS treatment whereas HSLF and HSLF-HFLS diets resulted in increased milk lactose content in comparison to the control. In multiparous group, HSLF treatment increased milk lactose content in comparison to the control and HSLF-HFLS treatment. The milk response indicated an increase in the total nutrient intake, favouring improvements in cows of the digested nutrients, metabolizable energy (ME), and somatotropic axis for high milk production in HSLF and HSLF-LSHF treatments, compared to the control. Normally, arrangements in somatotropic axis of dairy cows in early lactation result in the prioritisation of energy and nutrients, absorbed from digestion in the liver and stored in body reserves, towards the milk synthesis. Further research is required to evaluate the effect of energy levels and sources on the somatotropic axis in dairy cows that sustains not only the milk synthesis but also a rapid restoration of body reserves and an early fertility success (i.e. postpartum health, uterine regression, resumption of oestrous cycles, conception and embryo/pregnancy survival). Also, other energy sources (i.e. oat, barley, wheat, canola and rapeseed) and their combinations on milk responses of cows grazing on other irrigated forages (i.e. cynodon, digitaria, paspalum, etc.) produced in Western Cape warrant further investigation.

Postpartum energy levels and sources on ovarian follicular dynamics and oestrous activity of Holstein cows

Results on ovarian follicular dynamics and oestrous activity showed that no differences were observed between nutritional treatments in terms of the dimensions of the ovaries and the preovulatory follicle as well as the number of follicles within different classes in lactating dairy cows. However, grazing cows receiving 12.6 kg/day of concentrates in the HSLF and HSLF-LSHF treatments recorded a higher number of total follicles, indicating an improvement in the follicular growth, in comparison to those on 7 kg/day of concentrate in the control group. This response indicated an increase in the total nutrient intake, supporting an enhancement in available nutrients and EB status, in the HSLF and HSLF-LSHF treatments in comparison to the control. However, poor oestrous expression and an inability to easily detect oestrus are of concerns when using the artificial insemination (AI) technique in the reproductive management of dairy cows. Future research needs to investigate the influence of different inclusion levels and sources of energy nutrients that enhance the viability and the quality of the oocyte ovulating from the dominant follicle and improve the intensity and length of the oestrous expression in dairy cows. Also, types and levels of different dietary energy nutrients on the pregnancy rates with a large number of cows under field conditions and longer postpartum periods [i.e. 305 days in milk (DIM)] require further investigation.

Postpartum energy levels and sources on reproductive performance of Holstein cows

Results of reproductive performance showed improvements in the proportions of cows pregnant at 150 DIM in grazing primiparous and multiparous cows fed 11.6 and 12.6 kg/day of concentrate in HSLF and HSLF-LSHF treatments, compared to their counterparts being fed a control concentrate at 7 kg/day in the control, respectively. Higher inclusion level of concentrates in HSLF and HSLF-LSHF treatments improved the total nutrient intake and ME in dairy cows, and resulted in an improvement in pregnancy rates in comparison to the control. However, embryonic/pregnancy loss is the single biggest factor reducing calving rate in dairy cows. Further research is required to investigate different sources and inclusion levels of energy nutrients on maternal (i.e. progesterone level, oviductal and uterine environment, placental implantation, etc.) and gestational (i.e. production of interferon tau for pregnancy recognition, embryonic and foetal growth, etc.) factors that improve embryonic/pregnancy survival in dairy cows until the next calving. Also, the effects of other energy sources (i.e. oat, barley, wheat, canola and rapeseed) and their combinations on

fertility traits of cows grazing on other irrigated forages (i.e. cynodon, digitaria, paspalum, etc.) produced in Western Cape need to be investigated.

Late prepartum energy level on the live weight changes, milk production and fertility traits in the subsequent lactation period of Holstein cows

Results showed that late prepartum diets had no effect on milk yield and milk composition of Holstein cows during the subsequent lactation. Although primiparous cows on the prepartum partial total mixed ration (pTMR) diet had a significantly shorter interval from calving to first service compared to those on the prepartum oat hay diet, the conception rate at 120 DIM was similar for cows in both parity groups. Successful prepartum nutritional strategies should minimize health and rumen upsets and promote vigorous appetites after calving, resulting in a rapid increase of DMI and improvement in EB status. Different prepartum feeding periods and nutritional strategies involving different energy types and levels to stimulate postpartum metabolic and hormonal responses that benefit fertility, while sustaining milk production of dairy cows, warrant further investigation.

To conclude:

It can be concluded that feeding concentrates differing in energy levels (low *vs* high) and sources (starch *vs* fat) to postpartum dairy cows in a pasture-based system improved plasma metabolite profiles and LW, production responses, ovarian function and reproductive performances. These enhancements are likely related to dietary composition and levels of total energy intake, controlling the profile of nutrients absorbed in the gastro-intestinal tract (GIT) and their hepatic partitioning to various body tissues for metabolic requirements of dairy cows. This modulation probably influenced the hypothalamic-pituitary-ovarian-uterus axis through improved nutritional status, while optimizing the carry-over effect of improved ME into fertility success and sustaining high milk production. Such similar fertility responses between the HSLF and HSLF-LSHF treatments suggested that starch level in the HSLF diet were neither marginal nor harmful to affect negatively the conception rate of dairy cows, as reported in *in vitro* systems. Above all, improvements in milk production and conception rates of dairy cows with HSLF and HSLF-LSHF treatments have obvious positive biological effects to dairy farmers in comparison to the control. These biological effects include the decrease in fertility related culling and replacement of cows in the herd on one hand and the increase in herd size, cow longevity, lifetime milk production and financial return on the other hand. However, feeding two fibre-based diets differing in levels (low *vs* high) of NFC and protein in the late prepartum period showed no improvements in either milk responses or proportions of cows pregnant in the subsequent lactation. The lack of a significant difference of

prepartum diet effects on milk production and pregnancy success can be related to the prepartum feeding period and the relatively low number of animals per treatment used in the study. The reproduction process is complex and multifactorial in dairy cows. It is imperative to design in dairy cows an integrative approach that focuses on genetic selection, prepartum and postpartum nutrition, reproductive management, rumen milieu and animal health, farm management, environment, and cost of production. Such integration of these factors should collectively support not only the improvement of milk production and the early success of biological events associated with the establishment and the survival of pregnancy, but also the financial return and the sustainability of the dairy enterprise.